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SIXTH REPORT ON PLAGUE INVESTIGATIONS IN INDIA

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THE

JOURNAL OF HYGIENE

PLAGUE SUPPLEMENT I

SIXTH REPORT ON PLAGUE INVESTIGATIONS IN INDIA

ISSUED BY

THE ADVISORY COMMITTEE

APPOINTED BY THE SECRETARY OF STATE FOR INDIA,
THE ROYAL SOCIETY AND THE LISTER INSTITUTE

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Cambridge
at the University Press
1912

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VOLUME XI DECEMBER, 1911 Supplement

REPORTS ON PLAGUE INVESTIGATIONS IN INDIA.

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XL. Major GEORGE LAMB, M.D. Glasg., I.M.S.[1]

With Portrait (Plate I).

By the death of Major Lamb the Indian Medical Service has lost one of its ablest and most esteemed members, and science has been robbed of one of her most ardent devotees.

Dr Lamb was a man of great scientific attainments and, although but 41 years of age, had already achieved a reputation which is by no means confined to India. His contributions to science are as well known and as highly appreciated by scientific men throughout the world as by his colleagues in the Service. His early death, at a period when his judgment had matured but his imagination and enthusiasm remained unimpaired, is a serious loss to the small band of earnest scientific investigators in India, and one which will not easily be made good.

Lamb was educated at the Glasgow High School, whence he proceeded to the University of Glasgow in 1886. He had a brilliant university career, and on graduating in 1890 receiv[illegible] Bruton Memorial Prize as the most distinguish[illegible] ...ved the r. On taking the degree of M[illegible] ...ded the Bellahoust[illegible] ...raduate of his ...ng resident a...point[illegible] ...nary under ...r, he ...for Cameron and ...he became Demonstrator of Anatomy under ...his position he relinquished in 1893 when he entered ...an Medical Service, obtaining the first place in the competitive ...rance examination. At Netley he obtained most of the prizes available, and at the conclusion of the course again took the first pl...

At Netley, Lamb met Sir Almroth Wright, who was then Professor of Pathology at the Army Medical School. The direction of his interests towards pathology was due, in large measure, to Prof. Wright's influence. Lamb owed much to the inspiration gained from this teacher, and was always pleased to express his indebtedness and to insist with charming enthusiasm on the great stimulus Wright's teaching had been to him.

[1] The substance of this notice first appeared in the *British Medical Journal*, 29 April, 1911.

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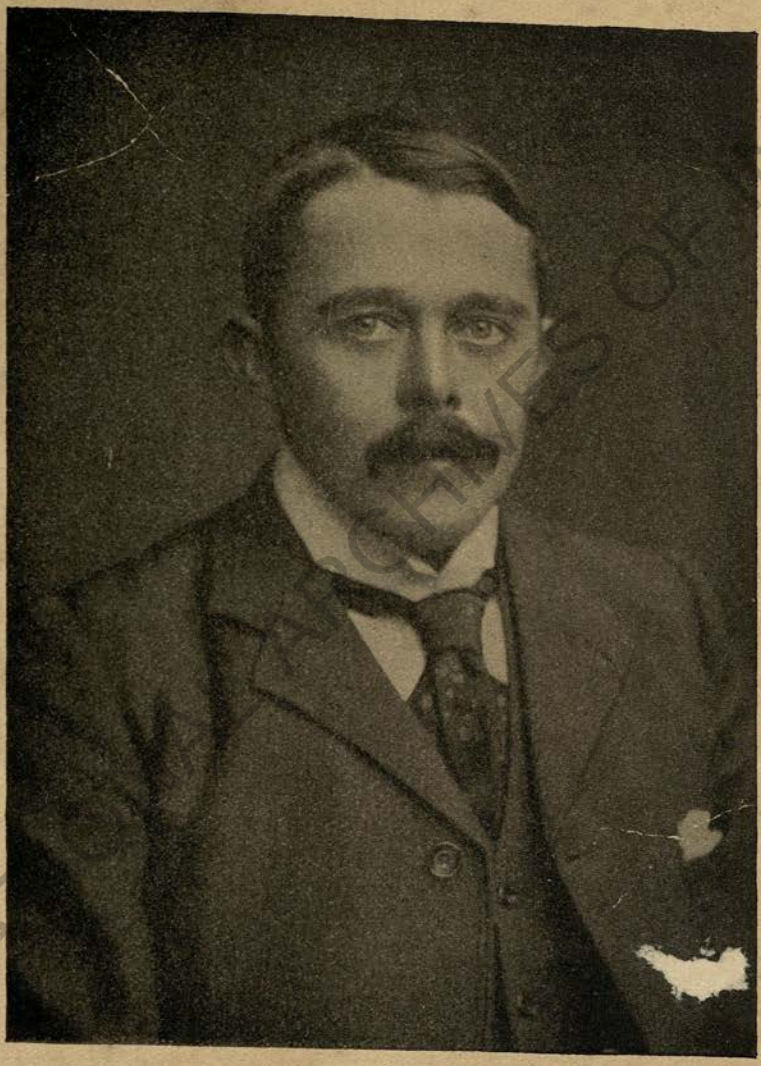

George Lamb

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In April 1894 Lamb proceeded to India, and for the next three years was employed in military service, mostly on the North-West Frontier, where he took part in the Waziri expedition. He could hardly have conformed to all the conventional ideas of a military surgeon, but he was very popular with his brother officers, and greatly esteemed by the troops under his medical care. These consisted for the most part of men recruited from the hill tribes—wild creatures whose morals and ideals differed no doubt from average European standards, but for whom, nevertheless, he entertained a considerable measure of respect. On the Frontier, especially under active service conditions, Lamb encountered life stripped of most of its affectations, an experience which is always educative and salutary, and especially so to one who has just spent seven years of academic existence. Although often at the time chafing to be again at scientific work, he was wont to look back upon his three years spent as a military surgeon on the Frontier without regret and with evident enjoyment.

In 1897 he returned to Europe on six months' leave, which he spent in study at the Pasteur Institute in Paris. On the termination of his leave, he was delighted to find that he had been selected by the Government of India to proceed to the Royal Army Medical School at Netley for further training in pathology and bacteriology, and to assist his former teacher, Prof. Wright, in the teaching work of the department of pathology. This happy arrangement lasted for eighteen months, and Lamb always regarded it as the most joyful time of his life. The admiration he had conceived for Wright, when a student at the Army Medical School, rapidly developed into the warmest affection, with the closer intimacy which he now enjoyed with his chief. The teaching duties were neither arduous nor continuous, and the major portion of his time and energy was available for research. The conditions in the laboratory were most congenial. The other assistants were Major Semple (now Sir David Semple) and Captain Birt (now Colonel Birt), with both of whom he collaborated in researches and formed close friendships, which lasted throughout life. Whilst at Netley, Lamb published three valuable papers dealing with various aspects of immunity, which showed that the brilliant student had become a capable investigator.

Returning to India in 1899, he became assistant to Prof. Haffkine, at that time Director of the Bombay Bacteriological Laboratory at Parel. In addition to numerous duties connected with his post, Lamb now commenced a series of investigations into the action of Indian

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snake venoms, for which he is justly renowned. These researches, which at first dealt with the nature and physiological action of the poisons produced by the different serpents of India, were later concerned with the production, properties and standardization of anti-venomous serums, and brought to light many facts of fundamental importance to the doctrine of immunity. The results appeared in twenty papers published between 1899 and 1907, partly in the *Scientific Memoirs* of the Government of India and partly in the *Lancet*. These researches, which involved the analysis of the action of the different poisons upon the various mechanisms of the animal body, necessitated the employment of very various physiological and pathological techniques. They were carried out in laboratories in India which were by no means sumptuously equipped for such inquiries. Nevertheless, they are models of insight and accuracy, and form a monument which will endure in the annals of scientific achievement. At the commencement of these inquiries Lamb was himself bitten by a cobra whilst manipulating the reptile in the laboratory, and narrowly escaped with his life.

It was about this time that I first met Lamb. His earlier researches on snake poison had led to a correspondence between us, and in 1901 it was arranged that we should meet in Bombay. I recall with vivid distinctness our first meeting. It was in one of the quaint laboratories transformed out of old Government House, Parel, which had previously been a Portuguese Jesuit seminary. It smelt strongly of teak, of which the fittings were constructed. I was enchanted by the simple frankness and geniality which contributed so greatly to the charm of his personality, and within a few minutes we seemed to be old friends. It was, however, his extraordinary keenness which impressed me most. Making every allowance for the intensification of the impression by the background—the climate of Bombay does not conduce to the manifestation of acute mental alertness and enthusiasm—Lamb struck me as the keenest man I had ever met. I remember we spent most of the night in a half-naked condition discussing snake poisons, and that it was one of the most entertaining conversations I have ever enjoyed.

Dr Lamb left Bombay in 1903, and spent the next few years at Kasauli, where he was assistant to Colonel Semple at the Pasteur Institute of India. Shortly before leaving Parel he married Patricia Liston, the sister of his old friend and colleague, Major Glen Liston.

The effect of strenuous work in Bombay had been very trying, and he left that picturesque city regretting only its glorious harbour, for he was an enthusiastic sailor-man, and his only recreation was sailing his

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Tom-Tit. Kasauli, being situated on the summit of one of the foot-hills of the Himalayas, enjoys a delightful climate, excellent for children, and addiction to laboratory work may be indulged with as little injury to health as in this country. The isolation, too, was congenial both to Mrs Lamb and himself, and he looked forward to much quiet happiness and increasing opportunities for scientific work. Although at Kasauli the work of the Pasteur Institute, where about 1000 patients were annually treated for rabies, made greater demands upon his time than had the duties of the post at Parel, this was more than compensated for by the improved climatic conditions. The snake-poison work was continued and experiments on the standardization of antityphoid vaccines and other researches incidental to the work of the institute were also carried out.

Before leaving Bombay, Lamb had discovered the existence of Mediterranean or Malta fever in India, and was inclined to believe that many of the obscure cases of fever amongst native troops, hitherto indiscriminately ascribed to malaria, were really cases of this disease. This view was warmly combated in many quarters. At Kasauli, however, he had the opportunity of studying some suspicious outbreaks amongst troops in the Punjab, and succeeded not only in justifying his contention, but showed that the disease was brought about by the consumption of infected goat's milk, as had recently been ascertained to be the case in Malta by the Mediterranean Fever Commission.

In 1905, Colonel Semple having been transferred to the new Central Institute for Research recently built at Kasauli, Dr Lamb succeeded to the position of Director of the Pasteur Institute. At the same time he gained his majority by accelerated promotion on the ground of his distinguished services.

He had not long held this responsible position, however, when the Advisory Committee asked the Government of India to place the services of Major Lamb at their disposal. The Government of India could not at first see its way to comply with this request. The committee was convinced, however, that Lamb was essentially the man to be the senior member of the Commission, and after a good deal of importunity their request was acceded to, with results which more than justified their expectations.

The Council of the British Medical Association, recognizing the importance of Major Lamb's share in the results of the work of the Commission, last summer awarded him the Stewart Prize for the most

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important contribution made within the last few years to our knowledge of the spread of a disease.

In this appreciation of Major Lamb the scientific side of his career has been more particularly represented. His death will also be a serious grief to a large number of friends and acquaintances, who came to love and admire him as a man, and among those who will mourn his loss most acutely will be his old bearer, who had faithfully served him since he came to India, and whom I last saw tending with the same pride and care the little sahibs of the next generation.

C. J. M.

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XLI. SOME RECENT OBSERVATIONS ON RAT FLEAS.

I. *The fleas common on rats in different parts of the world.*

CHICK and Martin[1] have recently published a valuable summary of our knowledge on this subject and give full references to the literature. The chief data may be shortly summarised as follows:—

Locality	Fleas examined	Percentage of total fleas belonging to each species					
		Xenopsylla cheopis	*Ceratophyllus fasciatus*	*Ceratophyllus anisus*	*Ctenopsylla musculi*	*Ctenophthalmus agyrtes*	Other species
India	891,000	100	trace	—	—	—	—
Australia	2,000	63	11	—	24	—	2
San Francisco	31,000	28	58	—	8	—	6
Japan	8,000	18	—	46	24	—	12
Mediterranean	13,500	58	25	—	11	—	6
England	1,000	—	48	—	—	52	—

The proportionate figures represent only the relative prevalence of the different kinds in the exact circumstances under which the sample of rats and fleas was obtained. Some differences in locality may materially alter the flea population; thus at Marseilles and in Japan it has been clearly shown that *X. cheopis* is much more frequent in connection with shipping than in more truly indigenous foci. The general prevalence of *X. cheopis* about the world is therefore probably exaggerated by the figures given, which apart from India and England mostly refer to seaports.

Other fleas, which are not primarily rat fleas, also occur on rats: cat (*Ct. felis*), dog (*Ct. canis*), and human (*P. irritans*) fleas have been found, usually in small numbers, by many observers and several other species have been recorded. In Italy as many as 25 % or 30 % of the fleas found on rats have been observed to be cat and dog fleas, and in German East Africa a fifth was the fowl flea (*Echidnophaga gallinacea*). In

[1] *Journal of Hygiene*, Vol. XI. (1911), p. 122.

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this locality *Xenopsylla scopulifer* (*X. brasiliensis*) also occurred pretty freely[1]. No evidence has been obtained that, other conditions being the same, the flea population on *Mus rattus* is different from that on *M. decumanus*.

II. *The occurrence of* Xenopsylla cheopis *in England.*

Chick, Martin and Rowland have found that the fleas on wild country rats in Suffolk, Hertfordshire and Devonshire are about equally divided between *C. fasciatus* and *Ct. agyrtes*. In Suffolk, Martin and Rowland got 584 fleas off 568 rats, the highest prevalence in any one locality being 3½ fleas per rat[2]. A single specimen of *X. cheopis* was taken at Plymouth some years since[3], but otherwise the species has not been found in this country. The following data therefore regarding the fleas found on *M. decumanus* trapped in Guy's Hospital, London, are of some interest[4]. Locality *B* is distant rather less than 100 yards from locality *A*.

Locality A.

Date	Rats caught	*C. fasciatus*	*X. cheopis*
1. 2. 11	1 big	0	0
3. 2. 11	6 young	18	1
6. 2. 11	1 ,,	9	1
14. 2. 11	1 big	2	0

Locality B.

10. 3. 11	1 big	0	0
17. 3. 11	1 young	0	30
21. 3. 11	2 ,,	0	75
22. 3. 11	1 ,,	3	49
22. 3. 11	1 ,,	1	3
23. 3. 11	2 ,,	0	73
12. 4. 11	1 big	0	9
13. 4. 11	2 ,,	1	66
26. 4. 11	1 young	1	36
30. 8. 11	1 ,,	1	41
31. 8. 11	1 ,,	3	18
31. 8. 11	1 big	2	14

[1] By an error in tabulation Chick and Martin enter this flea as having been recorded by the Commission from Poona (Vol. x. p. 524); the record refers to *P. irritans*.

[2] *Reports to the Local Government Board on Public Health and Medical Subjects*, New Series, No. 52 (1911), p. 49.

[3] Giles, *Entomol. Monthly Mag.* Vol. XLI. (1905), p. 139.

[4] *Guy's Hospital Gazette*, Vol. XXV. (1911), pp. 73, 318; *Entomol. Monthly Mag.* (2nd series), Vol. XXII. (1911), pp. 68, 113.

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Thus in locality *A*, 94 % were *C. fasciatus*, while in locality *B*, 97 % were *X. cheopis*. In all 24 rats yielded 457 fleas, an average of 19 fleas per rat.

III. *The biting of man by rat fleas.*

Chick and Martin[1] have made a number of interesting experiments on the readiness with which the various species of rat flea bite human beings and animals. Each flea was given an opportunity of biting for two minutes. The results may be summarised as follows:—

		Number of experiments	Percentage of positive results
C. fasciatus:	man	517	60
,,	rat	101	58
,,	rabbit	32	72
Ct. musculi:	man	111	4
,,	mouse	11	82
Ct. agyrtes:	man	98	none
,,	rat	19	58

C. fasciatus therefore bites man quite readily, while *Ct. musculi* and *Ct. agyrtes* do so hardly or not at all. A number of observations have shown that the race of *X. cheopis* occurring in London bites men greedily, even immediately after being taken off a rat (see Vol. VII. p. 472, VIII. p. 239). Thus off a freshly-caught rat seven *C. fasciatus* and 13 *X. cheopis* were obtained with chloroform at 9.45 a.m. At 12.30 they had recovered from the anaesthetic and were given the opportunity of biting the human arm: within five minutes all the *C. fasciatus* and 11 of the *X. cheopis* bit.

IV. *The seasonal prevalence of rat fleas.*

Gauthier and Raybaud[2] have published numerical data with respect to the various sorts of fleas taken off rats at Marseilles at different times of the year. Their figures for *X. cheopis* are as follows, together with the average mean monthly temperature and humidity:—

Month	*X. cheopis*	Temperature	Humidity
January	39	44° F.	68 %
February	6	47	64
March	5	50	61
April	12	55	60
May	2	61	59
June	2	68	57
July	21	72	54
August	32	71	57
September	50	67	63
October	39	59	69
November	26	51	71
December	24	45	70

[1] *Journal of Hygiene*, Vol. XI. (1911), p. 129.

[2] *C. R. Soc. Biol.* Vol. LXVIII. (1910), pp. 196, 198.

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The numbers on which the flea figures are based are not very large and the results are irregular; they indicate however sufficiently clearly a great increase of *X. cheopis* during the autumn. Plague used to prevail in the south of France at that season. For the other species of fleas their figures are still smaller, and the results very irregular. On the whole however they suggest that the abundance of *C. fasciatus* and *Ct. musculi* varies in much the same way.

Tidswell[1] has published similar figures for Sydney, based on the examination of 26,744 rats of which 177 were plague infected and off which 1926 fleas were obtained (62 % being *X. cheopis*).

Month	Fleas per 1000 rats			Average mean monthly temperature	Average mean monthly humidity
	X. cheopis	*C. fasciatus*	*Ct. musculi*		
January	57	26	43	72° F.	70 %
February	50	4	34	71	73
March	112	11	26	69	75
April	97	2	13	65	78
May	31	1	11	58	77
June	28	2	13	54	79
July	21	5	10	52	77
August	5	0	10	55	74
September	50	3	40	59	70
October	8	20	27	63	68
November	2	3	8	67	68
December	3	10	11	70	68

The plague season here is January to July with a maximum in March, April and May, so that there is good correspondence with the season of maximum prevalence of *X. cheopis*. Though all three species of flea seem to take part in the small autumnal increase, the great spring prevalence affects *X. cheopis* more than the others. This might, if confirmed, constitute an important epidemiological consideration and would reduce the significance of the fact that *C. fasciatus* bites men so readily.

In Japan[2], Kitasato has found that the absolute and relative abundance of *X. cheopis* is much increased during the autumn, *i.e.* during the plague season.

[1] *Report of the Government Bureau of Microbiology* for 1909 (Sydney, 1910), p. 20.

[2] *Trans. Bombay Med. Congress*, 1909, p. 93.

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XLII. PRELIMINARY OBSERVATIONS ON THE PROTECTIVE AND CURATIVE VALUE FOR RATS OF THE SERUM OF A HORSE IMMUNISED WITH A TOXIC NUCLEO-PROTEIN EXTRACTED FROM THE PLAGUE BACILLUS.

By SYDNEY ROWLAND, M.A., M.R.C.S.
Of the Lister Institute.

In a previous report (Vol. x. p. 536) I have dealt with a method of extracting from the plague bacillus a substance or substances which appear to be responsible both for the acute toxic action and immunising properties of killed plague bacilli. I have since investigated the properties of the serum of a horse immunised with this solution. In this inquiry I have had the advantage of the assistance of Dr A. T. MacConkey, Head of the Serum Department of the Lister Institute, who kindly undertook the supervision of the immunisation of the horse and to whom I desire to express my thanks.

For the purposes of the experiment a horse was selected and immunisation commenced on June 26, 1909. The material injected into the horse consisted of nucleo-protein dissolved in dilute saline prepared as explained in my previous report, where it was designated solution *B*. The method of immunisation adopted was as far as possible that usually employed in the Serum Department of the Lister Institute for the preparation of diphtheria antitoxin. The table gives the dates and doses administered intramuscularly. Unfortunately owing to lack of toxin the intervals towards the end of immunisation were longer than were absolutely necessary.

The local reaction after inoculation was almost identical with that observed after inoculation of diphtheria toxin. Some tenderness was present and swelling and oedema varying in amount, with transitory

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constitutional disturbance and little temperature reaction. There was no tendency to abscess formation or to the huge hard swellings of long duration which are not infrequently the result of injection of unfiltered cultures.

TABLE.

Date	Dose in mgs.	Date	Dose in mgs.	
June 26	0·01	July 26	28·0	
28	0·02	Aug. 3	42·0	
July 3	0·1	10	80·0	
7	0·2	24	100·0	Sample bleeding
9	0·4	Sept. 9	15·0	taken.
13	0·8	Oct. 26	30·0	
15	1·6	Nov. 13	60·0	
17	1·6	20	120·0	
20	3·5	Dec. 8	240·0	
22	7·0	29		Bled 8 litres.
24	14·0			

Neutralisation of the toxic extract (solution B) by means of the serum.

The first point to settle was whether the serum contained any substance capable of neutralising the toxin contained in solution *B*, *i.e.*, the toxin with which the horse had been immunised.

Method. The method used to investigate this point was to mix a known number of lethal doses of the toxin (M.L.D. = 0·1 mg. nucleoprotein) with a known volume of the serum, to leave the mixture at 35° C. for 30 minutes and then to inject it subcutaneously into rats and observe the result. If the rat lived, it was considered that the toxin had been neutralised.

Experiment 1[1].

Rat	No. of L.D. of toxin	Serum in c.c.	Result
1	10	0·1	−
2			−
3	25	0·1	+ 1 day
4			+ 1 day

1/10th c.c. of serum neutralises 10 lethal doses of toxin but not 25.

[1] Throughout these records − or 0 = lived, + = died.

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Experiment 2.

Rat	No. of L.D. of toxin	Serum in c.c.	Result
1	5·0	0·1	–
2			–
3			–
4	7·5	0·1	–
5			–
6			–
7	10·0	0·1	–
8			+ 1 day
9			+ 1 day
10	12·5	0·1	+ 1 day
11			+ 2 days
12			+ 2 days
13	15·0	0·1	+ 1 day
14			+ 1 day
15			+ 1 day
16	17·5	0·1	+ 1 day
17			+ 1 day
18			+ 1 day

The serum used in this experiment was from a second bleeding 5 days after the first bleeding. 1/10th c.c. of this serum neutralised 7·5 lethal doses of toxin.

In these experiments (1 and 2) the dose of serum was kept constant and the dose of toxin varied; in the next series the dose of toxin was kept constant and the dose of serum varied.

Experiment 3. *Estimation of the amount of serum required to neutralise* 10 *lethal doses of toxin B.*

Rat	Serum in c.c.	Result
1	0·08	+ 1 day
2		+ 1 day
3		+ 1 day
4		–
5		–
6	0·1	+ 1 day
7		+ 1 day
8		+ 1 day
9		–
10		–
11	0·12	–
12		–
13		–
14		–
15		–
16	0·14	–
17		–
18		–
19		–
20		–

10 lethal doses toxin were neutralised (*qua* rat) every time by 0·12 c.c. serum and four out of ten times by 0·1 and 0·08 c.c. serum.

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It having been shown that the amount of serum necessary to neutralise 10 lethal doses of the toxin, when mixed with it before injection into a rat, lies between 0·1 and 0·12 c.c., we have now to determine whether this ratio still holds when dealing with a greater number of lethal doses of the toxin. For this purpose 100 lethal doses of toxin were mixed with the quantities of serum shown in the following table: the mixture stood for one hour at 37° C. and was then injected into rats.

Experiment 4.

Rat	No. of L.D. of toxin	Serum in c.c.	Result
1	100	2	Survived
2			
3		1·75	,,
4			
5		1·5	,,
6			
7		1·25	,,
8			

From this experiment it is evident that the value of the ratio is maintained.

We conclude then *that the serum of this horse can neutralise the toxin in solution B.*

As affording some information on the progress of immunisation we give here the result of a test made, about half-way through the immunisation process, of a sample bleeding on August 24th.

Experiment 5.

Rat	L.D. toxin	Serum in c.c.	Result
1	102	1·0	+ 1 day
2			+ 1 day
3	68	1·0	+ 2 days
4			+ 3 days
5	34	1·0	−
6			−
7	3·4	1·0	−
8			−

At this time 1 c.c. of serum neutralised 34 lethal doses of toxin, compared with between 85 and 100 at the time of the final bleeding.

Effect of Yersin serum on the toxin in solution B.

It is necessary to compare with this serum that obtained by Yersin's method of immunisation, *i.e.* the ordinary anti-plague serum on the market, which is prepared by the inoculation of dead, and later of living, cultures of the plague bacillus.

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Experiment 6. *Estimation of amount of Yersin serum (prepared at the Lister Institute) necessary to neutralise* 10 *lethal doses of toxin.*

Rat	Serum in c.c.	Result
1	2·0	+ 1 day
2		+ ,,
3		+ ,,
4		+ ,,
5		+ ,,
6	1·0	+ ,,
7		+ ,,
8		+ ,,
9		+ ,,
10		+ ,,
11	0·5	+ ,,
12		+ ,,
13		+ ,,
14		+ ,,
15		+ ,,
16	0·1	+ ,,
17		+ ,,
18		+ ,,
19		+ ,,
20		+ ,,

No evidence of any antitoxic action is shown by this serum.

Similar results were obtained with two other samples of Yersin serum (Negress and Mayboy).

In the case of two other samples (Kit and Corbally) 0·5 c.c. of the serum neutralised 10 lethal doses of toxin and 0·25 c.c. serum failed to do so.

Prophylactic value of the serum against infection.

The serum of the horse immunised with solution *B* is capable, in common with some of the sera obtained by the usual methods of immunisation, of protecting against a subsequent inoculation of virulent plague. In experiment 7 (p. 16), the rats received the amount of serum indicated, and the standard test dose of virulent culture was given on the following day.

Curative value in plague infection.

So far attention has been confined to the antitoxic and anti-infectious properties of the serum, and the question remaining to be answered is:—has the serum any effect when administered to an animal that is already suffering from plague?

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Experiment 7.

Dose of serum	Rat	Result
1·0 c.c.	1	0
	2	0
	3	0
	4	0
	5	0
	6	0
	7	0
	8	0
	9	0
	10	0
0·1 c.c.	11	+ 4 days
	12	0
	13	0
	14	0
	15	0
	16	0
	17	0
	18	+ 3 days
	19	0
	20	+ 4 days
0·01 c.c.	21	+
	22	+
	23	+
	24	+
	25	+
	26	+
	27	0
	28	+
	29	+
	30	0

For this purpose the following experiment was performed. Sixty rats were inoculated with the usual dose of a virulent living plague culture; ten of these were left as controls, of which seven died of plague. The remaining fifty were treated in batches of ten—five batches. Six hours after inoculation with the living culture, batches 2, 3, 4, 5 and 6 received 0·5 c.c. serum subcutaneously on the opposite side of the body to that on which the living plague culture had been inoculated. Six hours later batches 3, 4, 5, and 6 received a second and similar dose; six hours later batches 4, 5, and 6 a third; six hours later batches 5 and 6 a fourth, and six hours later batch 6 received a fifth dose.

The results of this treatment are shown in experiment 8.

This table shows:

(1) That the infectivity of the test virulent culture was well up to the standard (Vol. x. p. 544).

(2) That taking all the rats together the death rate had been reduced from 80% to 18%.

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Experiment 8.

Batch	Rat	Treatment after infection. Serum c.c. 6 hrs.	12 hrs.	18 hrs.	24 hrs.	30 hrs.	Result	Average
1	1	...	...	...	...	...	+ 3 days	3·5 days
	2	...	...	...	...	...	+ 3 days	
	3	...	...	...	...	...	+ 3 days	
	4	...	...	...	...	...	+ 4 days	
	5	...	Controls.	No treatment.		...	+ 4 days	
	6	...	...	...	...	...	+ 4 days	
	7	...	...	...	...	...	+ 4 days	
	8	...	...	...	...	...	0	
	9	...	...	...	...	...	0	
	10	...	...	...	...	...	0	
2	1	0·5	...	...	...	...	+ 4 days	5 days
	2	0·5	...	...	...	...	+ 6 days	
	3	0·5	...	...	...	...	0	
	4	0·5	...	...	...	...	0	
	5	0·5	...	...	...	...	0	
	6	0·5	...	...	...	...	0	
	7	0·5	...	...	...	...	0	
	8	0·5	...	...	...	...	0	
	9	0·5	...	...	...	...	0	
	10	0·5	...	...	...	...	0	
3	1	0·5	0·5	...	...	...	+ 9 days	6½ days
	2	0·5	0·5	...	...	...	+ 4 days	
	3	0·5	0·5	...	...	...	0	
	4	0·5	0·5	...	...	...	0	
	5	0·5	0·5	...	...	...	0	
	6	0·5	0·5	...	...	...	0	
	7	0·5	0·5	...	...	...	0	
	8	0·5	0·5	...	...	...	0	
	9	0·5	0·5	...	...	...	0	
	10	0·5	0·5	...	...	...	0	
4	1	0·5	0·5	0·5	...	...	0	
	2	0·5	0·5	0·5	...	...	0	
	3	0·5	0·5	0·5	...	...	0	
	4	0·5	0·5	0·5	...	...	0	
	5	0·5	0·5	0·5	...	...	0	
	6	0·5	0·5	0·5	...	...	0	
	7	0·5	0·5	0·5	...	...	0	
	8	0·5	0·5	0·5	...	...	0	
	9	0·5	0·5	0·5	...	...	0	
	10	0·5	0·5	0·5	...	...	0	
5	1	0·5	0·5	0·5	0·5	...	+ 6 days	6 days
	2	0·5	0·5	0·5	0·5	...	+ 6 days	
	3	0·5	0·5	0·5	0·5	...	0	
	4	0·5	0·5	0·5	0·5	...	0	
	5	0·5	0·5	0·5	0·5	...	0	
	6	0·5	0·5	0·5	0·5	...	0	
	7	0·5	0·5	0·5	0·5	...	0	
	8	0·5	0·5	0·5	0·5	...	0	
	9	0·5	0·5	0·5	0·5	...	0	
	10	0·5	0·5	0·5	0·5	...	0	
6	1	0·5	0·5	0·5	0·5	0·5	+ 9 days	10 days
	2	0·5	0·5	0·5	0·5	0·5	+10 days	
	3	0·5	0·5	0·5	0·5	0·5	+11 days	
	4	0·5	0·5	0·5	0·5	0·5	0	
	5	0·5	0·5	0·5	0·5	0·5	0	
	6	0·5	0·5	0·5	0·5	0·5	0	
	7	0·5	0·5	0·5	0·5	0·5	0	
	8	0·5	0·5	0·5	0·5	0·5	0	
	9	0·5	0·5	0·5	0·5	0·5	0	
	10	0·5	0·5	0·5	0·5	0·5	0	

50 treated — 9 deaths

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(3) That in those cases where death of the treated rats occurred the length of life after injection was prolonged from 3–5 days to 10 days.

The next experiment was to test the maximum length of time that could be allowed to elapse between infection with plague and the inoculation of serum, consistent with the survival of the animal.

For this experiment 30 rats received the standard dose of living virulent culture. The rats were divided into three batches of 10 each. Batch 1 were not treated and remained as controls. Batch 2 received 0·5 c.c. serum 24 hours after infection. Batch 3 received 0·5 c.c. serum 48 hours after infection. The results of this experiment are shown in table 9.

Experiment 9.

Batch	Rat	Treatment	Days after infection 1	2	3	4	5	6	7	8	9	10	
2	1	0·5 c.c. serum 24 hours after infection	...	...	...	...	...	...	...	...	...	...	Survived.
	2		...	...	...	...	...	...	...	...	...	...	Survived.
	3		...	...	...	...	...	...	...	...	...	...	Survived.
	4		...	...	...	...	...	...	...	...	...	...	Survived.
	5		...	...	...	...	...	...	...	...	...	...	Survived.
	6		...	...	...	...	...	...	...	...	...	...	Survived.
	7		...	...	...	...	...	...	...	...	...	...	Survived.
	8		...	...	...	...	...	...	...	...	...	...	Survived.
	9		...	...	...	...	+						
	10		...	...	...	+							
3	1	0·5 c.c. serum 48 hours after infection	...	...	+								
	2		...	...	...	...	...	...	...	...	...	...	Survived.
	3		...	...	+								
	4		...	...	...	...	...	...	...	...	...	...	Survived.
	5		...	...	+								
	6		...	...	+								
	7		...	+									
	8		...	...	...	...	...	...	...	...	...	...	Survived.
	9		...	...	...	...	...	...	...	...	...	...	Survived.
	10		...	...	...	...	...	...	...	...	...	...	Survived.

The serum therefore saves the lives of a sensible proportion of infected rats even if its administration is delayed for as long as 24 hours after inoculation. It should be noted that the untreated rats begin to die on the second day.

In the case of the untreated batch, all the rats died; this must be taken as indicating that the virulence of the test culture was well up to the standard (80% deaths) and does not indicate any increase in virulence of the culture or diminution in resistance of the rats.

A comparative test was made between the curative value of Yersin's serum as prepared by the Lister Institute and the serum prepared by inoculation of solution B. In both cases ten rats received, 6 hours

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after inoculation, 0·5 c.c. of serum hypodermically on the opposite side to the infecting dose of virulent culture. Ten control untreated rats showed the virulence of the test culture to be up to the standard.

Experiment 10.

Batch	Rat	Treatment	Result. Days after infection 1	2	3	4	5	6	7	8	9	10	
1	1		...	...	...	+							
	2		...	...	...	...	+						
	3	0·5 c.c.	...	...	+								
	4	Yersin	...	...	...	...	+						
	5	serum	...	...	...	+							
	6	6 hours	...	...	...	...	...	...	...	...	...	...	Survived.
	7	after	...	...	...	+							
	8	infection	...	...	...	...	...	...	...	...	...	...	Survived.
	9		...	...	+								
	10		...	...	+								
2	1		...	...	...	+							
	2		...	...	...	...	...	...	...	...	...	...	Survived.
	3	0·5 c.c.	...	...	...	...	...	...	...	...	...	...	Survived.
	4	new	...	...	...	...	...	...	...	...	...	...	Survived.
	5	serum	...	...	...	...	...	...	...	...	...	...	Survived.
	6	6 hours	...	...	...	...	...	...	...	...	...	...	Survived.
	7	after	...	...	...	...	...	...	...	...	...	...	Survived.
	8	infection	...	...	...	...	...	...	...	...	...	...	Survived.
	9		...	...	...	...	...	...	...	...	...	...	Survived.
	10		...	...	...	...	...	+					

It has thus been demonstrated that the *antiplague serum prepared by immunising horses with a solution of the nucleo-protein from the plague bacillus prepared as described in the previous report possesses not only antitoxic and anti-infectious properties, but that it also excercises in rats a markedly curative effect when injected 24 hours after the animals have been infected with virulent plague bacilli.*

These results are most encouraging and further research is being directed towards obtaining a still more powerful serum.

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XLIII. SECOND REPORT ON INVESTIGATIONS INTO PLAGUE VACCINES.

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With 1 Diagram.

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In a former report (this *Journal*, Vol. x. p. 536) some of the contents of plague bacilli which had been killed by chloroform were extracted by two methods: (1) simple digestion of the organisms with saline, (2) treatment of the moist bacteria with enough anhydrous sulphate of soda to combine with all the water present, freezing and

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thawing the mixture by changing the temperature from 18° C. to 37° C.[1], filtering off the bacterial deposit at 37° C. and subsequent extraction of the bacteria with water.

The two solutions so obtained possess much the same chemical properties. Both contain one or more kinds of nucleoprotein, accounting for about two-thirds of the total nitrogen in the extract. As regards their physiological properties, however, there is a striking difference. The first extract, in the previous report called solution A, has only a small degree of toxicity for rats and, by the method employed (this *Journal*, Vol. x. p. 545), its immunising value was shown to be negligible. On the other hand, the second extract, obtained after treatment with sodium sulphate and called solution B, was fatal to rats, 0·05 to 0·1 mg. killing them in 18 hours: doses of 0·001 to 0·01 mg. afforded substantial protection, the latter amount reducing the mortality after the inoculation of a standard dose of plague culture from 80 % to 10 %. The toxic and immunising properties always accompanied the nucleoprotein throughout the various chemical manipulations to which it was subjected. The residue left after treatment with sodium sulphate and extracting with water was shown to be devoid of acute toxicity and immunising properties.

I. The Action of Chloroform.

The cause of the discrepancy in physiological activities of these two chemically similar extracts was not clear at the time of my previous report. I was inclined to believe that the toxic and immunising properties resided in some substance other than the nucleoprotein which for some unexplained reason was extracted together with it only after treatment with the sodium sulphate. Since then I have been able to clear up the mystery and at the same time to explain the different conclusions arrived at by Pick and myself with regard to the effect of chloroform upon the antigen of the plague bacillus.

It is stated by Pick (Kraus and Levaditi's *Handbuch*, Bd. I. p. 353) that chloroform destroys the antigen of the plague bacillus and in the first of these reports I explained that I had not been able to confirm this statement but had found that a toxic solution possessed of good immunising power could be prepared from organisms that had been

[1] Na_2SO_4 changes to $Na_2SO_4 10OH_2$ between these temperatures. At 37° C. the mixture exists as an emulsion of bacteria in a saturated solution of Na_2SO_4, at 18° C. as a hard rocky mass of Glauber's salt and dry bacteria.

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killed by chloroform. The explanation of these contradictory statements is afforded by the following experiments which show (1) that chloroform combines with some substance in the extract giving rise to a relatively atoxic modification; (2) that this compound is reversible; and (3) that a ready method of reversing the combination and of reinstating the toxicity is afforded by the use of anhydrous sodium sulphate.

Exp. 1. Sixty grammes of "sulphated" bacilli[1] were taken and divided into two portions of 30 grammes each. The sulphate was removed from both portions by adding just sufficient water to dissolve it at 37° C. and filtering. The pasty masses of bacilli left on the filter were treated as follows:

part 1 was extracted for 3 hours in chloroform water,
part 2 was extracted for 3 hours in distilled water: both at 37° C.

Both portions were then filtered and the nucleoprotein in the filtrate determined as usual. Both extracts contained the same quantity of nucleoprotein. The lethal dose of the nucleoprotein of the first solution was 2 mg., of the second 0·1 mg. The chloroform water extract although containing the same amount of nucleoprotein only possessed $\frac{1}{20}$th the toxicity of the water extract. This might be explained on the assumption that the nucleoprotein was not the active agent and the following experiment supports this interpretation.

Exp. 2. The same experiment was repeated with the modification of adding chloroform to the water extract after the completion of the extraction. The results are given below and show that the toxicity of the solution was unaltered. The degrading effect of chloroform on toxicity is thus greatest at the moment of extraction.

Details of experiments 1 and 2 are given in Table I.

In my previous report it was shown that the toxic and immunising properties of the extract prepared by the "sulphate process" followed so closely the content of nucleoprotein that it was difficult to believe that they belonged to some merely adherent substance and not to the nucleoprotein itself.

The following experiments, which show that the action of chloroform

[1] Wherever possible, in the course of this work, the various extracts have been made from the same batch of "sulphated" bacilli, a large stock of which, several kilogrammes, was prepared, thoroughly mixed and kept in the ice box. The method of preparation of this powder of "sulphated" bacilli is given in the last report in full. Briefly it is the washed surface growth from agar set in Roux flasks, mixed with anhydrous sulphate of soda and ground to a fine powder. Once prepared it apparently preserves its properties indefinitely and is a most convenient means of ensuring a constant supply of plague bacilli.

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on the nucleoprotein is reversible, completely explain the apparent inconsistency of the results of experiments 1 and 2 with this view.

EXP. 3. A quantity of an emulsion of organisms was prepared and a few cubic centimetres of chloroform added to the flask so that some remained free and undissolved at the bottom of the flask. The emulsion

TABLE I. *Experiment* 1.

Toxicity of the extract made in chloroform water.			*Toxicity of the water extract.*		
Dose, mgs.	Rat	Result	Dose, mgs.	Rat	Result
1·7	1	*+ 1 day	1·7	1	+ 1 day
	2	0		2	+ 1 day
0·8	3	0	0·8	3	+ 1 day
	4	0		4	+ 1 day
0·4	5	0	0·4	5	+ 1 day
	6	0		6	+ 1 day
0·2	7	0	0·2	7	+ 1 day
	8	0		8	+ 1 day

* The symbol + is used throughout this report to indicate the death of the animal, 0 its survival.

Experiment 2. *Toxicity of the water extract to which chloroform was added.*

Dose, mgs.	Rat	Result
1·7	1	+ 1 day
	2	+ 1 day
0·8	3	+ 1 day
	4	+ 1 day
0·4	5	+ 1 day
	6	+ 2 days
0·2	7	+ 2 days
	8	—

TABLE II.

Experiment 3. *Determination of toxicity.*

	(1) Emulsion in chloroform water		(2) Same emulsion after pumping out the chloroform	
Dose, mgs.	Rat	Result	Rat	Result
1·0	1	0	1	+ 1 day
	2	0	2	+ 1 day
0·8	3	0	3	+ 1 day
	4	0	4	+ 1 day
0·6	5	0	5	+ 1 day
	6	0	6	+ 1 day
0·4	7	0	7	+ 1 day
	8	0	8	+ 1 day
0·2	9	0	9	+ 1 day
	10	0	10	+ 1 day

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was divided into two parts. Both parts were incubated at 37° C. for 6 hours. Part 1 was set aside, while part 2 was exhausted by the Fleuss pump for some hours until no smell of chloroform was noticeable. Both parts were left till the next day when they were filtered and the nucleoprotein content and toxicity determined (see Table II).

The lethal dose of the pumped out portion was 0·2 mg., possibly less, while that from which the chloroform had not been so removed was greater than 1 mg. The amount of nucleoprotein was the same in each.

EXP. 4. A quantity of organisms killed by chloroform were mixed with sulphate of soda in the usual way and the sulphate removed by dissolving with water at 37° C. As we have seen, this process yields organisms free from chloroform and in such a condition that the nucleoprotein with its toxic and antigenic properties can be extracted with water from the bacilli. In this case chloroform was added to the water with which the paste of organisms was extracted. The chloroform water extract was comparatively toxic, although it contained the usual amount of nucleoprotein. On pumping out the chloroform, however, the solution was again rendered toxic to the usual degree (M.L.D. = 0·1 mg.). After this extraction the organisms were again mixed with sulphate which in its turn was removed. The paste of organisms left after this second sulphating was extracted in water and a small yield of nucleoprotein again obtained, of which the lethal dose was as usual about 0·1 mg. (see Table III).

These experiments show that the atoxic nucleoprotein extracted by means of chloroform water can be rendered toxic by the simple process of removing the chloroform in a vacuum. There is, therefore, no reason to suppose, as was done in my previous report, that the nucleoprotein extracted by water from the organisms killed by chloroform (nucleoprotein A in the last report) is not the same substance as that extracted from similar material after sulphating (nucleoprotein B of last report). The nucleoprotein A is in combination with chloroform, and for this reason has lost its toxic properties which, however, can be reinstated by removing the associated chloroform with the air pump. It is further clear that one of the effects of the sulphate of soda used in the process is to remove this chloroform from its loose combination with the nucleoprotein.

This removal of chloroform by the sulphate of soda appears to be due to evaporation of the chloroform following on the withdrawal of all the water by the anhydrous salt to form $Na_2SO_4 10H_2O$.

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TABLE III. *Toxicity determinations.*

Dose mgs.	Chloroform water extract: Rat	Chloroform water extract: Result	Chloroform water extract after pumping out the chloroform: Rat	Chloroform water extract after pumping out the chloroform: Result
2·5	1	0	1	+ 1 day
	2	0	2	+ 1 day
1·25	3	0	3	+ 1 day
	4	0	4	+ 1 day
0·5	5	0	5	+ 1 day
	6	0	6	+ 1 day
0·25	7	0	7	+ 1 day
	8	0	8	+ 1 day

Dose, mgs.	Second water extract: Rat	Second water extract: Result
1·1	1	+ 1 day
	2	+ 1 day
0·56	3	+ 1 day
	4	+ 1 day
0·28	5	+ 1 day
	6	+ 2 days
0·05	7	+ 3 days
	8	+ 3 days

Why the effect of chloroform upon the toxicity and immunising power of the nucleoproteins should be so different, according to whether it is brought into play at the moment of extraction or subsequently (as in Experiment 1), still remains unexplained. It may well be that the composition of these nucleoproteins at the moment of soaking out from the recently killed bacillus is somewhat different to that which obtains afterwards. Nucleoproteins are bodies with great possibilities of combination with other proteins and there is no evidence that they exist as such inside the living cell.

An interesting point with regard to the reversibility of the chloroform-nucleoprotein-toxin combination emerges from the study

TABLE IV.

Delayed mortality, using chloroformed nucleoprotein.

Dose, mgs.	Rat	Result
1·6	1	+ 1 day
	2	0
0·83	3	+ 8 days
	4	+ 8 days
0·4	5	+ 5 days
	6	+ 4 days
0·2	7	+ 27 days
	8	+ 10 days

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of the effect of this substance when inoculated into rats. The lethal dose (in this work the death of the animal in two days is regarded as determining the L.D.) is large (1 to 2 mg.) but there is observed a delayed mortality amongst the animals corresponding to a gradual dissociation of the compound after inoculation. Thus in one experiment the results in Table IV were obtained.

It will be a matter of interest to follow up this delayed toxic action in the body since its bearing on the problem of vaccination is apparent.

The effect of toluol on the toxicity and immunising properties of the nucleoprotein.

Toluol exerts but slight deleterious influence upon the activity of solutions of the nucleoproteins of the plague bacillus and was found to be the best preservative to use during their preparation. I tried therefore whether this substance could be advantageously employed instead of chloroform vapour to kill the bacilli.

Preliminary experiments were made to determine the time necessary to kill the organisms. For this purpose a good surface growth on agar in a Roux flask was used. The flask was inverted and a few cubic centimetres of toluol introduced. At the end of each quarter of an hour a whole loopful of the growth was withdrawn from the flask and inoculated on the surface of agar. In this way the following results (Table V) were obtained:

TABLE V.

Showing the presence or absence of growth in the tubes inoculated from the flask after exposure to toluol vapour for the times indicated.

Quarter hour intervals	Growth after		
	24 hours	48 hours	72 hours
1	+	+	+
2	+	+	+
3	+	+	+
4	–	+	+
5	–	+	+
6	–	+	+
7	–	–	–
8	–	–	–
9	–	–	–
10	–	–	–
11	–	–	–

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These preliminary experiments showed that toluol can be used as a germicide in the same way as chloroform (viz. inverting the Roux flask and introducing a few c.c. of the liquid into the flask, thus exposing the bacilli to the action of the vapour only), about one and a half hours being required to completely sterilize a Roux flask. Seventy-three Roux flasks were treated in this way. Of the emulsion obtained, one half was left in contact with toluol for two days, the other half was centrifugalised and washed as usual. After washing, the latter was sulphated in the ordinary way, and a water extract corresponding to solution B obtained; of this solution 0·2 mg. nucleoprotein was a lethal dose. The portion of the emulsion left in contact with the toluol was filtered, and of the fluid obtained the lethal dose was about 1·0 mg.

Toluol apparently behaves similarly to chloroform and forms a loose combination with the nucleoprotein, the combination being less toxic. By treatment with anhydrous sulphate of soda a more toxic product is obtained (Table VI).

TABLE VI.

Toxicity of toluol water extract.			*Toxicity of water extract from toluol-killed and sulphated organisms.*		
Dose, mgs.	Rat	Result	Dose, mgs.	Rat	Result
1·5	1	+ 3 days	4·0	1	+ 1 day
	2	0		2	+ 1 day
0·6	3	+ 3 days	2·0	3	+ 1 day
	4	0		4	+ 1 day
0·3	5	+ 3 days	1·0	5	+ 1 day
	6	0		6	+ 1 day
0·15	7	0	0·4	7	+ 1 day
	8	0		8	+ 1 day
			0·2	9	+ 1 day
				10	+ 2 days

II. On the Presence of a Hydrolytic Enzyme in the Plague Bacillus.

A quantity of plague bacilli were grown on agar in Roux flasks. They were killed by chloroform vapour and an emulsion made in salt solution: 8 % of the nitrogen of the emulsion was soluble in saturated tannic acid[1]. The emulsion was incubated under toluol at 37° C.

[1] This method of determining the progress of hydrolysis is due to Hedin (Hedin and Rowland, "Ueber ein Proteolitisches Enzym in der Milz," *Zeitschr. f. phys. Chem.* Bd. xxxii. p. 342). The precipitation of the undigested proteids is effected by the addition of an equal volume of a solution of tannic acid 7 % and acetic acid 5 %.

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After 24 hours 28 % of the total nitrogen was in a form which was not precipitated by tannic acid. In 6 days 38 % was reduced to this form, while in 10 days 50 % was so changed.

The experiment was repeated with the addition of 11 % sulphate of soda to the emulsion. In this case 38 % of the nitrogen was converted in 24 hours and 48 % after 10 days.

The solution contained 780 milligrammes of protein in 100 c.c. The quantities found after the lapse of the times indicated are given in Table VII.

TABLE VII.

Time	Protein found, mgs. per 10 c.c.
As prepared	78
18 hours	68
45 hours	49
65 hours	49
22 days	38

The diminution of the nitrogen precipitable by tannic acid and of the total proteins coagulable by heat runs parallel, from which it is concluded that the bulk of the protein hydrolysed by the enzyme is derived from the nucleoprotein. The great falling off in rate as the hydrolysis proceeds at the stage when less than 50 % of the total protein has been attacked, suggests either that the enzyme is readily destroyed, or that only a proportion of the protein is capable of hydrolysis by this means. The effect of the hydrolysis by this enzyme upon the toxicity and immunising power of the nucleoprotein extract will be dealt with directly (p. 35 below).

There exists, therefore, in the plague bacillus an enzyme capable of hydrolysing the protein. For the sake of comparison with a phenomenon which is better known, an experiment was made with some brewer's yeast under the same conditions. Here after 5 days 73 % of the nitrogen was converted, while in the presence of sulphate of soda (10 %) 59 % was changed.

The activity of this enzyme is therefore comparable with that of a proteolytic ferment of recognised power.

The question now arises, is this enzyme extracted by the methods employed in preparing an actively immunising solution by the sulphate process? A quantity of solution of nucleoproteins was prepared by dehydration with Na_2SO_4 and extraction in saline and examined in the same way. The percentage of nitrogen hydrolysed to the extent of being soluble in tannic acid mixture is shown in Table VIII.

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TABLE VIII.

The solution contained 7·8 mgs. of nucleoprotein per c.c.

Time	Percent. nitrogen not precipitable by tannic acid mixture after various intervals at 37° C.
As prepared	5·7
18 hours	29
45 hours	41·7
65 hours	44
22 days	56

Concurrently with this hydrolysis there was a loss in protein as estimated by boiling after acidulation with acetic acid.

III. The Effect of heating Solutions of the Nucleoprotein on their Immunising Power, Toxicity and the Activity of the Hydrolytic Enzyme contained therein.

As shown by Famulener and Madsen (*Biochem. Zeitschr.* Bd. XI. p. 186, 1908), and by Martin and Chick (*Journ. Physiol.* Vol. XL. p. 404, 1910), the effect of hot water upon certain antigens (vibrio-lysin, tetano-lysin) and upon proteins does not occur instantaneously when a particular temperature is reached. It is a time process in which heat merely plays the subsidiary part of an accelerator. The action has an extraordinary high temperature coefficient and the rate of the reaction is increased in some cases as much as twice per degree centigrade rise in temperature.

Famulener and Madsen studied the effect of hot water upon vibrio-lysin and tetano-lysin and were able to obtain data for the calculations of the antigen destroyed as time elapsed by test tube experiments.

In the case of plague antigen, however, *in vitro* experiments are not feasible and the number of animal experiments required to determine each point would be very costly. I have therefore been obliged to content myself with observations indicating the fall in antigenic power suffered by the minimal optimal immunising dose by heating for half an hour to various temperatures. The diminution in immunity conferred was indicated by the percentage of animals which survived a subsequent inoculation of 0·1 c.c. of the standard plague culture after they had received the same quantity of antigen solution heated at each temperature. What the relation between the percentage protected and

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proportion of antigen remaining after heating may be, is at present being determined.

As stated in my previous report (this *Journal*, Vol. x. p. 559), the protection afforded by a single dose of one-hundredth of a milligramme of the nucleoprotein and associated antigen is to protect from 80–90 % of the rats inoculated against a subsequent dose of virulent plague such as will kill 80 % of the control animals. The following table gives the protection afforded by a similar dose of nucleoprotein which had been heated to the temperatures named for half an hour.

TABLE IX.

Temp. to which antigen solution was heated (° C.)	No. of rats in series	Mortality	% Mortality
Unheated	19	2	11
55	20	6	30
60	36	18	50
64	39	21	54
65	38	30	79
Controls inoculated with test culture only	100	—	78

In the last series a precipitation took place which was filtered off, the clear fluid only being inoculated.

This experiment shows that the destruction of antigen is appreciable below 55° C. and that the rate increases slowly until 64° C. is reached. At 65°, at which temperature the nucleoprotein was precipitated[1], the whole of the antigen disappeared from the solution, for the mortality was the same as in the controls.

1. *The effect of heating upon the toxicity of solutions of nucleoprotein.*

A quantity of toxic nucleoprotein solution was prepared and heated in the water bath to various temperatures for half an hour. Table X below shows the experimental results of inoculating these heated solutions into rats.

The minimal fatal dose of this sample of the nucleoprotein was evidently just above 0·1 mg., for this quantity of the unheated material killed the two rats in two and three days respectively. Heating to 53° C. and 60° C. for half an hour reduced the toxicity to $\frac{1}{4}$th (0·4 mg. = M.L.D.) and $\frac{1}{8}$th (0·8 to 1 mg. = M.L.D) respectively. The observations at 56° C. only give an upper limit.

[1] The precipitation of the nucleoprotein by heat does not always occur at this temperature. The exact point is dependent upon the reaction and concentration of the solution.

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From the series at 53° C. and at 60° C. some idea of the acceleration of the destruction per degree rise of temperature can be obtained. Taking 0·4 mg. as the minimal lethal dose after heating to 53° C. for half an hour and 0·9 mg. as the mean value after heating to 60° C. for the same period, the relative rates of destruction are as 9 to 4 for a rise of 7° C. This gives a temperature coefficient of 3·2 per 10° C.

TABLE X.

The solutions were of the same reaction and nucleoprotein concentration and were heated to the temperatures for half an hour.

Dose, mgs.	Rat	Kept in ice box	53° C.	56° C.	60° C.
4·0	1	...	...	+ 1 day	...
	2	...	...	...	+ 3 days
2·0	3	...	+ 1 day	+ 1 day	...
	4	...	+ 1 day	+ 1 day	...
1·0	5	...	+ 1 day	+ 1 day	+ 3 days
	6	...	+ 1 day	+ 3 days	0
0·8	7	...	+ 1 day	...	+ 3 days
	8	...	0	...	0
0·6	9	+ 1 day	+ 1 day	+ 1 day	0
	10	+ 1 day	+ 1 day	+ 1 day	0
0·4	11	+ 1 day	+ 1 day	...	0
	12	+ 1 day	0	...	0
0·2	13	Very ill	...	...	...
	14	Very ill	...	...	...
0·1	15	+ 2 days	...	...	...
	16	+ 3 days	...	...	...

2. *The effect of heating solutions of the nucleoprotein upon their hydrolytic activity.*

Like other enzymes, the hydrolytic ferment in the plague bacillus is destroyed by heating its solution. It is impossible to properly determine the influence of temperature upon the rate of the destruction of the enzyme by hot water, because, so far, I have not succeeded in separating the enzyme from the "substrate." Some qualitative notion of the influence of hot water was, however, obtained by heating solutions of the nucleoprotein containing the enzyme for half an hour at various temperatures, and estimating the amount of non-precipitable nitrogen after two days' subsequent incubation at 36° C. (Table XI).

As this method does not give an estimate of the work done by the enzyme after heating irrespective of the increased work done by the enzyme during the heating, inasmuch as it is not possible to instantaneously bring the solution to the temperature required, the experiment

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was repeated with a series of controls in which the changed nitrogen was estimated immediately after the heating. The results are given in Table XII.

TABLE XI.

Temp. to which the solution was heated	Percent. nitrogen not precipitated by tannic acid mixture
Control (not heated)	36·8
51—52° C.	33·8
53—54	30·4
55—56	24·5
59—60	16·6
63—64	13·0

TABLE XII.

Temp. to which the solution was heated for 30 mins.	Amount of nitrogen in non-precipitable form	
	Determined at once	After two days
Control	10·9 %	49·5 %
39° C.	12·1	—
44	—	47·9
50	14·7	—
55	15·0	40·0
58	15·1	—
63·5	13·1	17·4

These determinations indicate that on heating for half an hour the temperature at which the first damage to the enzyme can be recognised is about 50° C.

3. *Comparison of the effect of heating the bacilli on the toxicity and immunising power of the extract subsequently obtained from them, with the effect of heat applied to the extract itself.*

A. *With regard to toxicity.*

For this experiment 100 Roux flasks of plague bacilli were prepared as usual, but instead of being chloroformed they were heated to 60° C. in the steamer. The steamer was first raised to this temperature, then opened, the flasks introduced and when it had again reached 60° C. the time was noted and heating continued for half an hour. The surface growth was washed off as usual in salt solution and the emulsion centrifugalised. The first washings, *i.e.* the salt solution that had been in contact with the bacilli for some two or three hours, in contrast to what occurred in the case of bacilli killed by chloroform, contained no

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nucleoprotein in solution. The paste of organisms was "sulphated" in the usual way, the mass left to cool and placed at 37° C. for the night. Next morning enough water at 33° C. to dissolve the sulphate was added and the whole placed on a hardened filter. The residue of organisms left on the filter was extracted with water. The suspension would not filter and rapidly became a slimy mass resembling sputum. It was accordingly centrifugalised and an opalescent fluid obtained. The amount of nucleoprotein obtained was equal to that usually yielded by similar treatment of chloroform killed, *i.e.* unheated, bacilli. The nucleoprotein obtained by this process was of about the same toxicity as that obtained after killing the bacilli with chloroform, 0·2 mg. being an acute lethal dose.

In view of the effects of heating the toxin already given, the fact that this solution was highly toxic indicates that there is a considerable difference in the effect produced by heating it in solution in water and heating it in the condition in which it occurs within the body of the bacillus.

TABLE XIII.

Lethal dose of extracted nucleoprotein heated to 55° C. *for half an hour.*

Dose, mgs.	Rat	Result
2·0	1	+ 1 day
	2	+ 1 day
1·0	3	+ 1 day
	4	+ 2 days
0·6	5	+ 1 day
	6	+ 1 day
0·4	7	0
	8	0

Lethal dose of an emulsion heated to 55° C. *for half an hour.*

Dose, mgs.	Rat	Result
2·0	1	+ 1 day
	2	+ 2 days
1·0	3	+ 1 day
	4	+ 3 days
0·5	5	0
	6	0

The difference between the effect of heat upon the toxicity of the extracted nucleoprotein and of the bacillus respectively was further studied by comparing the toxicity of the extracted nucleoprotein heated to 55° C. with that of organisms heated to the same temperature.

In order to institute a comparison between the toxicity of the whole bacilli and that of the extracted nucleoprotein it is necessary to know what proportion of the bacillus the latter comprises. From previous observations (this *Journal*, Vol. x. p. 536) it was found that the nucleoprotein constitutes about one-tenth by weight of the whole bacillus. The toxicity of the extracted nucleoprotein solution after heating to 55° C. for half an hour was found by experiment to be between 0·5 and

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1 mg. On the assumption that the effect of heat upon toxicity when the bacillus is heated as a whole is the same as when the toxic nucleoprotein is heated after extraction, the lethal dose of whole bacilli heated to 55° C. should be 6·0 mg. whereas it was about 1 mg. The protocols of this experiment are given in Table XIII.

The contrast is still more marked if a comparison be made between the toxicity of the nucleoprotein solution made from unheated organisms and a similar solution made from organisms that have been heated to 60° C. for half an hour, a temperature which we have seen reduces the toxicity of the nucleoprotein solution to one-ninth. At a temperature at which the toxicity of a solution in water is greatly reduced, only a small diminution occurs when the toxic nucleoprotein is in the cell, as the following experiment shows.

TABLE XIV.

Toxicity of nucleoprotein solution heated to 60° C. for half an hour.			*Toxicity of nucleoprotein solution prepared from organisms that had been heated to 60° C. for half an hour.*		
Dose, mgs.	Rat	Result	Dose, mgs.	Rat	Result
4·0	1	+ 5 days	0·8	1	+ 1 day
	2	0		2	+ 1 day
1·0	3	+ 3 days	0·4	3	+ 1 day
	4	0		4	+ 2 days
0·8	5	+ 3 days	0·2	5	+ 2 days
	6	0		6	+ 2 days
0·6	7	0	0·1	7	0
	8	0		8	0

The lethal dose of the toxin prepared from unheated organisms is about 0·1 mg.

I have at present no explanation to offer to account for the difference in the rate of destruction of the toxin according to whether it is heated whilst within the bacillus or after extraction. The very large effect of small changes in the concentration of hydrogen ions and variations in the concentration of salts upon the rate with which proteins are attacked by hot water recently described by Chick and Martin, *Journal of Physiol.* Vol. XL. p. 404, 1910, suggests a possible explanation. This subject is at present under investigation, because of the practical importance of any facts which may lead to a differential method of obtaining the antigen and toxin which are apparently bound up in the nucleoprotein.

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B. *With regard to immunising power.*

A comparison of the effect of heat upon the immunising properties of the nucleoprotein (1) whilst within the bacillus and (2) subsequent to extraction, indicates that under the former circumstances a similar diminution in rate of destruction occurs to that found in the case of toxicity.

An emulsion of organisms was prepared and killed by heating to 55° C. for half an hour. Of 34 rats that had received such a volume of this emulsion as contained 0·01 mg. of dry bacterial substance, 31 survived the subsequent test inoculation with plague. The amount of nucleoprotein contained in the quantity of organisms inoculated was (as was shown in the previous report, this *Journal*, Vol. x. p. 536) about one-tenth by weight, or 0·001 mg.

A second series of 20 rats were inoculated with ten times this quantity of extracted nucleoprotein that had been heated to 55° C. for half an hour precisely as was the emulsion used in the last experiment. Of these rats only 14 survived the subsequent test inoculation. There is here an increase of 21 per cent. of survivals in the case where the antigen had been heated inside the bacillus; and this increase in face of the fact that the dose of antigen was one-tenth as great[1].

IV. The Effect of Hydrolysis on the Toxicity and Immunising Properties of the Nucleoprotein Solution.

1. *Effect on toxicity.*

As mentioned on p. 27 above the watery or dilute saline extract of plague bacilli killed by chloroform vapour contains a hydrolytic enzyme. It had been noticed in the course of this investigation that the toxicity of an extract diminished day by day when kept at laboratory temperatures and in view of the fact recorded by Ruffer and Wilmore[2] that the endotoxin obtained from the dysentery bacillus is destroyed by digestion with pepsin, it was considered likely that this might be accounted for by the action of the enzyme. The following experiment shows that the

[1] That it was really one-tenth is a deduction based on the nucleoprotein content of the two vaccines. Until the relation between antigen injected and survivals has been worked out so as to afford a biological test for the amount of antigen, this is the only quantitative method of indicating the amount of antigen we possess.

[2] *British Medical Journal*, 1908, Vol. II. p. 1176.

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progressive hydrolysis of the nucleoprotein on incubating the solution at 37° C., described on p. 28 above, is accompanied by a loss of toxicity. This is shown in Tables XV and XVI below. The extract was slightly alkaline to litmus paper.

TABLE XV.

The original solution contained 7·8 mg. of nucleoprotein per cubic centimetre.

Time incubated at 37° C.	Lethal dose *
Fresh	0·05 c.c.
18 hours	about 0·4
45 hours	more than 1·0
65 hours	,, 7·0

* Portions removed from time to time to test toxicity were diluted 1 in 7·8.

TABLE XVI.

Protocols for determination of toxicity experiment in Table XV above.

Fresh solution			Solution incubated for 18 hrs.			Solution incubated for 45 hrs.		
Dose, c.c.	Rat	Result	Dose, c.c.	Rat	Result	Dose, c.c.	Rat	Result
0·3	1	+ 1 day	0·4	1	+ 1 day	1·0	1	0
	2	+ 2 days		2	0		2	0
0·2	3	+ 1 day	0·3	3	+ 2 days	0·8	3	0
	4	+ 2 days		4	0		4	0
0·1	5	+ 1 day	0·2	5	0	0·6	5	+ 1 day
	6	0		6	0		6	0
0·05	7	+ 1 day	0·1	7	0	0·4	7	0
	8	+ 2 days		8	0		8	0

Other determinations of the same point are here given (Tables XVII–XX). In these cases the solutions were kept at the temperature of the laboratory.

TABLE XVII.

The original solution contained 3·5 mg. of nucleoprotein per cubic centimetre.

Age	Lethal dose *
1 day	0·1 c.c.
24 days	1·0
54 days	more than 2·0
72 days	about 7·0

* Portions removed from time to time to test toxicity were diluted 1 in 3·5.

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TABLE XVIII.

Protocols of experiments in determination of toxicity in Table XII above.

Fresh solution.			Solution incubated for 24 days.			Solution incubated for 54 days.		
Dose, c.c.	Rat	Result	Dose, c.c.	Rat	Result	Dose, c.c.	Rat	Result
0·6	1	+ 1 day	4·0	1	+ 1 day	7·0	1	+ 1 day
	2	+ 1 day		2	+ 1 day		2	+ 1 day
0·4	3	+ 1 day	2·0	3	+ 1 day	3·5	3	+ 2 days
	4	+ 2 days		4	+ 1 day		4	+ 2 days
0·2	5	+ 1 day	1·0	5	+ 1 day			
	6	+ 2 days		6	+ 1 day			
0·1	7	+ 2 days	0·6	7	0			
	8	0		8	0			
			0·4	9	0			
				10	0			
			0·2	11	0			
				12	0			
			0·1	13	0			
				14	0			

TABLE XIX.

The original solution contained 5 mg. of nucleoprotein per cubic centimetre.

Age	Lethal dose *
Fresh	0·1 c.c.
4 days	0·4
17 days	about 4·0

* Portions removed from time to time to test toxicity were diluted 1 in 5.

TABLE XX.

Protocols of experiments in determination of toxicity, in Table XIII above.

Fresh solution.			Solution incubated for 4 days.			Solution incubated for 17 days.		
Dose, c.c.	Rat	Result	Dose. c.c.	Rat	Result	Dose, c.c.	Rat	Result
0·4	1	+ 1 day	0·4	1	+ 2 days	0·6	1	+ 2 days
	2	+ 1 day		2	0		2	0
0·3	3	+ 1 day	0·3	3	+ 3 days	0·4	3	+ 2 days
	4	+ 1 day		4	0		4	+ 2 days
0·2	5	+ 1 day	0·2	5	0	0·2	5	0
	6	+ 2 days		6	0		6	0
0·1	7	+ 2 days	0·1	7	0	0·1	7	0
	8	0		8	0		8	0
0·05	9	0						
	10	0						

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The accurate determination of the lethal dose of toxin demands a large number of animals. The results obtained in these three cases are sufficient to establish the fact that on hydrolysis there is a progressive loss of toxicity but are not sufficient to effect correlation between the extent of the loss and the work done by the ferment.

2. *Effect on the immunising power.*

As in the previous series of experiments the hydrolytic agent was the enzyme naturally occurring in the extract containing the nucleoprotein. The extract contained 8 milligrammes of nucleoprotein per cubic centimetre, and possessed a faintly alkaline reaction to litmus paper. It was kept at a temperature of 37° C. The amount of hydrolysis taking place in such an extract so kept and its effect upon the toxicity of nucleoprotein have already been considered (*vide* pp. 28, 36 above). We have now to determine what is the effect of the hydrolysis of the solution upon its immunising property.

In the first report (*vide* this *Journal*, 1910, Vol. x. p. 559), it was shown that a dose of extract containing 0·01 mg. nucleoprotein immunised from 80–90 % of rats against infection by a standard dose of the virulent living culture.

The source of this extract was an emulsion of organisms killed by chloroform vapour. We now know that during the time of exposure to chloroform, hydrolysis is proceeding, for it has been shown that it takes place in a chloroform water suspension. In dealing with the relation between hydrolysis and immunising power, it therefore follows that account must be taken of the influence of the time factor in the method of preparation.

As a preliminary an extract was prepared according to the original method which may be considered to have been hydrolysing for one day. The results of its inoculation into rats confirmed those obtained a year before, that a volume containing 0·01 mg. nucleoprotein protects 80–90 % the vaccinated animals.

TABLE XXI.

Protection afforded by extract hydrolysed for one day.

Dose, mg.	No. of rats inoculated	No. survived	Percentage surviving
0·01	19	17	89

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Preliminary trials having indicated that the effect of hydrolysis on the immunising properties of the extract was small it was allowed to autolyse at 37° C. for upwards of two months. After one month 14 rats were inoculated with a volume which contained 0·01 mg. of nucleoprotein at the beginning of this lengthy incubation. Of these animals 12 survived the subsequent test dose, *i.e.* 83 %. It follows then that the lysis of the nucleoprotein far from having destroyed its immunising power, had left it unaltered. Referring to what has already been said in this report as to the effect of lysis, it will be remembered that at this stage of the process some half of the available nitrogen has been transformed to a condition not precipitable by tannic acid mixture.

Having then failed to find that after one month's autolysis there was any diminution in the immunity conferred by the extract, corresponding to the reduction of toxicity, it was returned to the incubator and hydrolysis allowed to continue for a further period of six weeks. At the end of this time 15 rats received the same dose as before and of these 11 survived the subsequent standard infecting dose of living culture, giving a protection of 73 %.

The diminished percentage of protections may indicate that at this late stage of hydrolysis the immunising value of antigen is beginning to be affected, but in a series of 15 rats it is hardly outside the statistical error.

We are thus obliged to admit that the protection afforded by a solution of the nucleoprotein that was originally toxic is but slightly, if at all, diminished by allowing the solution to hydrolyse whereas this process has been shown to destroy the toxicity of the solution.

In addition to the experiments just mentioned where after one month and two and a half months the animals received of the autolysed extract doses whose volumes corresponded to 0·01 mg. of the original nucleoprotein content, other series of rats were inoculated with one-tenth this quantity of extract. Thus of 15 rats that received the dose equivalent to 0·001 mg. of the one month's hydrolysed extract, eight survived the subsequent test inoculation (53 %), and of 15 rats that received the dose equivalent to 0·001 mg. of the two and a half months' hydrolysed extract seven survived the test inoculation (46 %). Reducing the dose of the one month's hydrolysed extract to one-tenth thus reduced the percentage protection from 86 to 53 and reducing the dose of the two and a half months' hydrolysed extract to the same amount reduced the percentage protection from 73 to 46.

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In both cases the same reduction of the dose effected approximately the same reduction in the percentage protection.

This result affords corroborative evidence as to the relative accuracy of the percentages given by the experiment and used as a measure of the amount of immunising substance in the extract.

Putting these results in the form of a table we have:

TABLE XXII.

Effect of hydrolysis on immunising substance.

Time of hydrolysis	Dose, mg. nucleoprotein	No. of rats inoculated	No. of rats surviving	Percentage surviving
1 day	0·01	19	17	89
1 month	0·01	14	12	83
	0·001	15	8	53
2½ months	0·01	15	11	73
	0·001	15	7	46

The effect of hydrolysis under the influence of the hydrolytic enzyme normally present in extracts of the plague bacillus upon the immunising power presents analogies with Ruffer and Wilmore's (1908) results upon the hydrolysis of the endotoxin of the dysentery bacillus[1]. These authors found that by digesting for three days with pepsin and hydrochloric acid the toxicity was reduced to $\frac{1}{30}$th whilst the immunising value was not greatly, if at all, diminished. Gottstein (1908) also digested an emulsion of typhoid bacilli with pepsin and obtained a soluble product which he called "fermo toxin." This solution was toxic for guinea pigs and possessed of immunising action which according to this author was not impaired by boiling[2].

V. The Increase of Immunising Power as the Result of Hydrolysis.

The extract prepared by the method detailed in the last report has been considered as having been subjected to hydrolysis during the time occupied in the preparation. Roughly we may consider such an extract to have been hydrolysed for one day. When it is remembered that the rate of hydrolysis is greatest in the earlier stages, the importance of being able to compare the effect upon the toxicity and immunising value in these early stages becomes obvious.

[1] *British Medical Journal*, 1908, Vol. II. p. 1176.
[2] *Deutsches Arch. f. klin. Med.* Vol. XCIV. p. 255.

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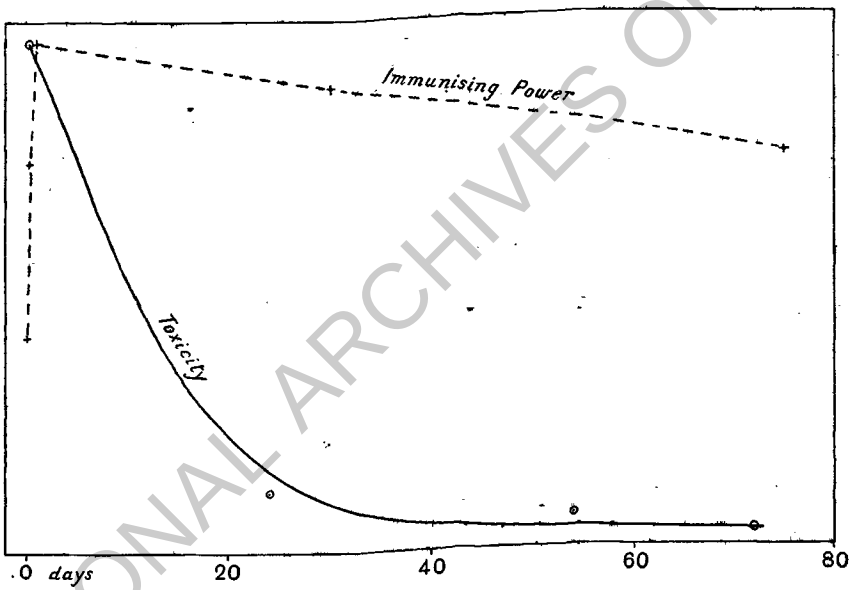
In the method as originally described the bacilli grown on the surface of agar in Roux flasks were submitted to the action of chloroform vapour for 12 hours; during the whole of this time, as we now know, hydrolysis was proceeding. If this time could be reduced or abolished we should be in the position of obtaining extracts which had been hydrolysed for only the reduced time or not at all.

An extract was therefore prepared so that the time of exposure of the organisms from which it was prepared—and so the time of hydrolysis—was reduced to *two* hours.



Of 33 rats inoculated with such a volume of this extract as contained 0·01 mg. nucleoprotein, 24 survived the subsequent standard infecting dose of virulent culture, = 73 %. This degree of protection is less than that obtained by a similar dose of what we have called the one day's hydrolysis extract (80–90 %), but taken by itself would hardly be significant.

To still further reduce the time of hydrolysis, an extract was prepared in which the use of chloroform as a killing agent was dispensed with, the bacilli being scraped off the surface of the agar

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alive, washed alive, and mixed alive with the anhydrous sulphate of soda, which killed them[1].

The only opportunity for hydrolysis to occur is in the final extraction of the sulphated organisms. This process occupied a short time only and took place at the temperature of the laboratory. The extract prepared from this material is therefore hydrolysed to a minimum possible under the circumstances.

Of 42 rats inoculated with a volume of this extract which contained 0·01 mg. of nucleoprotein, only 21 or 50 % survived the subsequent standard injection. This experiment is distinctly significant and indicates that the initial effect of hydrolysis is to *increase* the protection afforded by the same dose of nucleoprotein, and that a well hydrolysed extract is a better vaccine than one that has been submitted to the action of the enzyme to a less degree.

If we now put in the form of a table the figures representing the protection afforded by the various extracts (percentage rats surviving the standard infecting dose) and the figures expressing the hydrolysis time, it becomes clear that so far from hydrolysis diminishing the protective value, in the early stages of the process it actually increases it.

TABLE XXIII.

Relation between the protective value of the extracts and the amount of hydrolysis.

Hydrolysis time	Percentage rats protected by amount of extract originally containing 0·01 nucleoprotein
Fresh	50
2 hours	73
1 day	89
1 month	83
2½ months	73

From the above experiments the very important conclusion may be drawn that *the protective and toxic properties of the extract are distinct.* These may belong either to two different substances which are difficult to separate or be two properties attached to the same substance. So far neither has been observed apart from the nucleoprotein which they accompany throughout the various manipulations to which this substance

[1] As long as the bacilli are manipulated alive their contents do not escape into the solution. This is however a dangerous process and to anyone who wishes to repeat it the precaution of performing the mixture in an efficient draught cupboard is advised. During the final stages of the mixing the finely powdered material floats in clouds in a slight current of air.

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has been subjected. That, under the hydrolytic influence of the enzyme, the toxicity is greatly diminished whereas the protective power only manifests itself more markedly, is explicable on the assumption that both activities are proper to the nucleoprotein. If such be the case, the effect of hydrolysis suggests that the toxicity is bound up with the basic protein moiety and the immunity-conferring property with the nuclein part, as the nuclein complex is known to be highly resistant to hydrolytic agencies.

VI. Immunity against the Toxin.

Hitherto when referring to immunising properties the protective effect as discovered by a subsequent inoculation of living plague *only* has been considered. Since the anti-infectious antigen appears to be independent of the toxin it was legitimate to suspect that the immunity conferred upon my rats does not depend upon the development of antitoxin. Antitoxin, however, may be produced by the repeated injection in steadily increasing amount of the toxic nucleoprotein. This aspect of the subject is treated in another report (see this *Journal*; p. 11 above) but it is necessary to mention here that by treating a horse on much the same principle as is employed in the manufacture of diphtheria antitoxin a serum of quite encouraging antitoxic value was obtained.

The following experiments were made to determine whether any resistance to the toxic extract is conferred by a previous single injection of a sublethal dose of the toxic extract or of the atoxic product resulting from the autolysis of the same.

A series of rats were injected subcutaneously with doses varying from ·00005 mg. to ·05 mg. of toxic nucleoprotein. The highest dose is about half that quantity which usually kills a rat in 24 hours, and one-third of all the rats which received it died before the time for determining their resistance had arrived. Hence, those ultimately tested were to some extent selected rats. The extract was similar to that which repeated experience has shown will protect against a subsequent infection by the living organisms in doses of ·001 to ·01 (1910, p. 559).

The tests for resistance were made one month after the inoculation. In the first series of experiments the test dose varied from ·2 to 3·5 average M.L.D.'s. The results are set forth in Table XXIV and show a considerable degree of resistance to have been developed by the injection of the largest dose.

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TABLE XXIV.

Test of immunity conferred by a single dose of toxic nucleoproteid against a subsequent (one month) dose of toxic nucleoprotein.

Test doses M.L.D.'s	Rat	Immunising dose..0·05	0·005	0·0005	0·00005 mg.
3·5	1	+ +	+	+	+
	2	0 0	0	+	+
3·0	3	0 0	+	+	+
	4	0 0	0	+	+
2·5	5	0 0	+	+	+
	6	0 0	+	0	+
2·0	7	0 0	+	+	+
	8	0 0	+	0	+
1·5	9	0 0	0	+	+
	10	0	0	+	+
1·0	11	0	+	0	+
	12	0	0	0	+
0·5	13	0	0	0	+
	14	0	0	0	0
0·2	15	0	0	0	0
	16	0	0	0	0

The next series of experiments was devised to ascertain whether any similar antitoxic immunity was aroused by the nucleoprotein extract after it had been mostly deprived of toxicity by hydrolysis (see p. 36 above). The toxicity of the hydrolysed material was found to be at most one-tenth of the original. It must be remembered that the atoxic extract protects equally well against infection by the living organisms.

Table XXV below, in which the resistance developed with toxic and atoxic extracts is compared, shows that the large dose ·05 mg. of either confers a considerable degree of immunity, and from this experiment alone it is not possible to conclude that the toxic preparation possessed any advantage.

TABLE XXV.

Immunity against toxin by toxic and atoxic nucleoprotein respectively.

Immunising dose, mg...	0·05		0·005		0·0005	
	Toxic	Atoxic	Toxic	Atoxic	Toxic	Atoxic
Test dose 3 M.L.D.'s	Died 2 / Survived 6	Died 2 / Survived 6	Died 2 / Survived 2	Died 7 / Survived 1	Died 4 / Survived 0	Died 7 / Survived 1
Test dose 2 M.L.D.'s	Died 0 / Survived 11	Died 2 / Survived 5	Died 4 / Survived 2	Died 3 / Survived 5	Died 4 / Survived 2	Died 6 / Survived 2
Total survived	89 %	73 %	40 %	37 %	20 %	19 %

Controls 22 %.

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This result is contrary to expectation, and the matter is receiving further attention.

The conclusion that the toxicity and protective property of the extract are independent finds support from the results of experiments upon the protective value of the toxic nucleoprotein to which an anti-serum (*supra* p. 11) had been added.

A series of rats was inoculated with the serum alone, the toxic nucleoprotein alone, and with varying quantities of a neutralised mixture of the two. Altogether six series were prepared and two weeks later the degree of immunity developed by the animals was tested by the inoculation of the standard dose of the living virulent culture. The results are set forth in Table XXVI below:

TABLE XXVI.

Exp. 1. One-tenth lethal dose toxic nucleoprotein and serum to correspond.

	No. rats inoculated	No. protected	Percentage protected against subsequent infection
Serum alone	14	7	50
Toxic nucleoprotein alone	19	15	79
Mixture	15	12	80
Exp. 2. 10 neutralised lethal doses.	10	9	90
Exp. 3. 20 neutralised lethal doses.	8	8	100
Exp. 4. 50 neutralised lethal doses.	9	8	90

It will be seen from these results that the serum neutralised the toxicity without apparently affecting the substance to which protection is due. Exp. 1 in which the dose of nucleoprotein was only just optimal shows that the neutralisation of the toxicity has had no effect on the protective value of the solution. Thus the toxin may be put out of action either by neutralisation or by hydrolysis. In either case the particular antigen which is responsible for protection is, or becomes, free to act.

Working as a member of a large staff of an institute with many-sided activities, it is difficult to express individual thanks to the many of my colleagues who have helped me in the course of this investigation. I must however express my sense of obligation to Dr C. J. Martin, Director of the Lister Institute, whose constant advice and service have always been at my disposal.

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VII. Conclusions.

1. The toxic and immunising properties of the plague bacillus are attached to nucleoproteins which are soluble in water and can be extracted from the bacilli by suitable means.

2. Chloroform forms compounds with these nucleoproteins which are reversible.

3. These compounds with chloroform are relatively non-toxic and possess greatly diminished immunising powers against infection with the living organism but on removal of the chloroform the toxicity and immunising properties are regained.

4. Toluol forms similar compounds which behave in a similar manner to the analogous compounds with chloroform.

5. The effect of heat on the toxic and immunising powers of the nucleoproteins whilst in the bacillus is much less than on the toxic and immunising powers of the nucleoproteins after extraction from the bacillus. Solutions of nucleoproteins of full immunising and toxic power can be obtained from bacilli that have been heated to 60° C., whereas these properties are destroyed if solutions of the nucleoproteins exhibiting them which have been extracted from the bacilli are heated to this temperature.

6. The plague bacillus contains a proteolytic enzyme.

7. The extracts containing nucleoproteins in solution obtained under circumstances precluding as far as possible the action of this enzyme are more toxic than similar extracts obtained under circumstances allowing the enzyme to act.

8. In the early stages of the action of the enzyme on extracts containing nucleoproteins in solution exhibiting toxic and immunising powers, a fall in the value of the toxic power accompanied by a rise in the value of the immunising power occurs; by continued hydrolysis the toxicity practically disappears but the immunising power is not diminished.

9. Experiments show that some resistance against toxic extracts, as well as against infection, may be conferred by the inoculation of large doses of the same toxic extracts either in their original condition or in the atoxic modification produced by hydrolysis.

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XLIV. STATISTICAL INVESTIGATION OF PLAGUE IN THE PUNJAB. SECOND REPORT: ON THE CONNECTION BETWEEN PROXIMITY TO RAILWAYS AND FREQUENCY OF EPIDEMICS.

By M. GREENWOOD, Junr.

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With Map.

In my former report (Vol. x. p. 424) the suggestion was made that proximity to means of communication might have an important influence on the distribution of plague epidemics within a district, and that some such disturbing factor might have been specially influential in the district of Amritsar, which is characterised by the extreme irregularity of its epidemic distribution. The present report is occupied with an analysis of material bearing on this subject.

The train of ideas which led up to the suggestion just noticed was briefly as follows. The evidence collected by Major Lamb, together with his and my analysis, tended to support the conclusion that an outbreak of plague in a village was more likely to be caused by the importation of the disease from outside, in the last resort from some relatively constant centre of infection, than by the rekindling of embers which had remained inactive since a former outbreak. But, if this view were correct, it should follow that villages in frequent communication with centres of almost constant infection, such as the large cities, ought to show a heavier rate of recurrence than villages of the same size but more secluded. The circumstances which make for free intercommunication fall into two classes. In the first place, the better a village is served by means of transit in the shape of roads and railways, the greater, other things being equal, will be the intercourse between its inhabitants and the outside world. A second class of influences cannot however be neglected. The racial peculiarities of the inhabitants, their religious and social customs and, in some cases perhaps, their occupations may make a considerable difference. It

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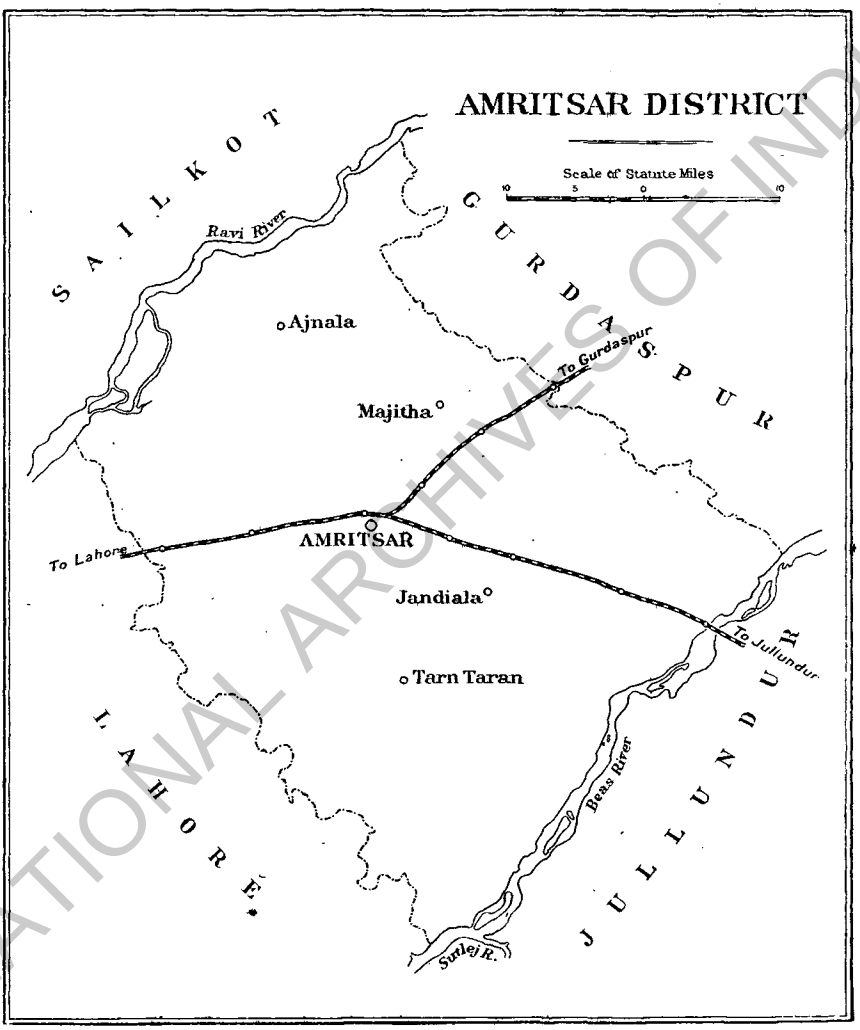
might be that village A although badly served by railways or other means of transit is inhabited by a class of people who are peculiarly prone to migrate, while village B, in an ostensibly more favourable situa-



tion, contains a predominantly sessile population. I draw attention to these points because it is necessary to bear in mind that the complete solution of epidemiological problems such as we have to face in connection with plague demands a knowledge of many elements not at present in our

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hands; I am here concerned merely with a single one of the influences which are at work and, although the reader will perhaps concede it to be an important one, it is by no means the only nor even the prime factor. The point I have actually investigated is the connection between proximity to railways and the frequency of plague epidemics in the three tehsils of Amritsar district. I first marked on the map all villages which were situated not more than two miles from one of the three lines of railway, *i.e.* the lines from Amritsar to Lahore, Gurdaspur and Jullundur (see Map). The number so included was 155, but this figure is probably not accurate within, say, half a dozen either way, owing to the difficulty found in identifying the key numbers on the maps with the corresponding villages in the data lists, a difficulty in part due to the synonymy which is presumably an effect of transliteration.

The list obtained included two municipal towns of large size, Amritsar city and Jandiala[1], which are much larger than the other villages and possibly not comparable in other respects; in the absence of specific mention to the contrary they are not taken account of in my analysis. The remaining villages are grouped in respect of plague history as shown in Tables I and II.

The first point to notice is that the distribution is unlike that of Amritsar district taken as a whole (Table III).

Had the latter distribution been valid for the selection we should have had the state of affairs indicated in Table III B.

TABLE I.

Summary of plague history of villages in Amritsar within two miles of the railway.

Number of infections	Number of villages
0	13
1	16
2	27
3	37
4	32
5	24
6	4
	153

[1] Re-measurement suggests that Jandiala is somewhat more than two miles from the railway.

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TABLE II.

Annual incidence of plague in the selected villages.

Year of infection	Number of villages
Infected in 1st year	9
,, 2nd ,,	73
,, 3rd ,,	79
,, 4th ,,	114
,, 5th ,,	77
,, 6th ,,	97

TABLE III.

A. *General summary of the whole Amritsar district.*

Number of infections	Number of villages
0	155
1	183
2	211
3	230
4	169
5	93
6	21
	1062

B. *Numbers of villages within two miles of the railway actually infected compared with the expected numbers calculated on the basis of the whole district.*

Number of infections	Calculated number of villages	Actual number of villages
0	22·33	13
1	26·36	16
2	30·40	27
3	33·14	37
4	24·35	32
5	13·40	24
6	3·03	4
	153·01	153

It will noticed that the selected villages exhibit a disproportionately large number of multiple infections and an unduly small number of unaffected and once affected members. What we have to determine is whether this difference is statistically significant or capable of explanation as a mere error of random sampling. The first question that arises is as to whether the difference is an effect of size variations, since we know that villages of large size are more likely to show multiple infections

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than are small ones. Tables IV and V show the size distributions of the selection (inclusive of municipal towns) and of the whole district; Table V repeats the actual figures for the selection together with the calculated figures on the supposition that the selection is a random sample in respect of size. Testing in the usual way we find $P = \cdot 15$

TABLE IV.

Population of villages within two miles of the railway (inclusive of two municipal towns).

Population	Number of villages	Population	Number of villages
0— 300	24	3000—3300	1
300— 600	46	3300—3600	0
600— 900	25	3600—3900	1
900—1200	18	3900—4200	0
1200—1500	16·5	4200—4500	0
1500—1800	13·5	4500—4800	0
1800—2100	5	4800—5100	0
2100—2400	1	5100—5400	0
2400—2700	1	5400—up	3
2700—3000	0		155·0

TABLE V.

Populations of all villages in Amritsar District.

Population	Number of villages	Population	Number of villages
0— 300	248	3000—3300	7·5
300— 600	299	3300—3600	5
600— 900	194·5	3600—3900	4
900—1200	105·5	3900—4200	4
1200—1500	69·5	4200—4500	6
1500—1800	47·5	4500—4800	1
1800—2100	35	4800—5100	2
2100—2400	13	5100—5400	0
2400—2700	8	5400—up	6
2700—3000	6·5		1062·0

and, although the small groups at one end of the series tend to worsen the fit, it must be admitted that the selection contains an appreciably too small proportion of little villages, a fact which is favourable to a large number of multiple infections. In order therefore to decide whether the incidence of plague upon the villages within two miles of the railway is really excessive we must proceed further with our analysis.

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Tables VII and VIII were next prepared and are self-explanatory. The reason for confining myself to villages not containing more than 2100 inhabitants was the small number and scattered distribution, in respect of size, which distinguish the larger villages. Table IX was then prepared; its method of construction will be plain from the following example.

TABLE VI.

Expected distribution of size in the selected villages on the basis of the returns for the whole district.

Population	Actual number	Calculated number
0— 300	24	36·20
300— 600	46	43·64
600— 900	25	28·39
900—1200	18	15·40
1200—1500	16·5	10·14
1500—1800	13·5	6·93
1800—2100	5	5·11
2100—2400	1	1·90
2400—2700	1	1·17
2700—3000	0	0·95
3000—3300	1	1·09
3300—3900	1	1·31
3900—4500	0	1·46
4500—5100	0	0·44
5100—etc.	3	0·88
	155·0	155·01

P (13 groups) = 0·15.

TABLE VII.

All Amritsar villages with populations up to 2100.

Population groups	Number of epidemics							Totals
	0	1	2	3	4	5	6	
0— 300	105	71	44	20	5	3	0	248
300— 600	43	75	81	70	23	7	0	299
600— 900	4	22	53	64	41·5	8	2	194·5
900—1200	2	10	15	29	31·5	16	2	105·5
1200—1500	0	1	11	18	25	11·5	3	69·5
1500—1800	0	1	5	7	15	18·5	1	47·5
1800—2100	1	1	2	13	7	10	1	35
Totals	155	181	211	221	148	74	9	999

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TABLE VIII[1].

All selected villages with populations up to 2100.

Population groups	Number of epidemics							Totals
	0	1	2	3	4	5	6	
0— 300	9	6	6	2	0	1	0	24
300— 600	4	7	13	16	4	2	0	46
600— 900	0	1	4	9	9	1	0	24
900—1200	0	1	3	5	6	3	0	18
1200—1500	0	0	1	2	7	4·5	2	16·5
1500—1800	0	1	0	0	4	8·5	0	13·5
1800—2100	0	0	0	1	0	3	1	5
Totals	13	16	27	35	30	23	3	147

Let us take the case of villages thrice infected. The chance of a village between 0–300 being three times affected is (Table VII) $\frac{20}{248}$, and therefore in 24 villages (Table VIII) we should expect $\frac{20\times 24}{248}$ instances. Similarly the contribution of the villages between 300 and 600 should be $\frac{70}{299}\times 46$. The total number should be

$$\frac{20\times 24}{248}+\frac{70\times 46}{299}+\frac{64\times 24}{194\cdot 5}+\frac{29\times 18}{105\cdot 5}+\frac{18\times 16\cdot 5}{69\cdot 5}+\frac{7\times 13\cdot 5}{47\cdot 5}+\frac{13\times 5}{35}=33\cdot 7.$$

Testing for goodness of fit, one finds $P=0\cdot 10$. The agreement is not close as it is, but the test is really too favourable to the chance of agreement for the following reasons. The totals (Table VII) upon which the prediction is based include the villages within two miles of the railway. But these latter actually form 14·7 per cent. of all the villages, hence some degree of approximation to the calculated values was only to be expected. A fairer test of differentiation seems to be to compare the actual distribution with that calculated on the basis of the villages which were more than two miles from a railway. For this purpose, Table X was prepared by subtracting the entries in Table VIII from the corresponding values in Table VII. From this table, the results given in Table XI were obtained by the process just illustrated. The value of P for this table shows that the agreement is considerably worse, that the selection exhibits too high a proportion of villages frequently infected and too low a proportion of villages rarely plague-stricken. All these results point to the conclusion that villages within reach of a railway are not a random sample of the whole of the villages. One further test may be mentioned.

[1] After the analysis had been completed another village was satisfactorily identified and should have figured in the selection. As no appreciable difference would be made in the results the tables have not been re-worked.

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TABLE IX.

Number of epidemics calculated for the selected villages on the basis of the whole district, attention being paid to size. For villages with populations up to 2100.

Number of epidemics	Actual number of villages	Calculated number
0	13	17·75
1	16	23·49
2	27	30·14
3	35	33·67
4	30	25·71
5	23	14·50
6	3	1·73
	147	147·00

$P = 0{\cdot}10$.

TABLE X.

Number of epidemics in villages more than two miles from the railway with populations up to 2100.

Population groups	Number of epidemics							Totals
	0	1	2	3	4	5	6	
0— 300	96	65	38	18	5	2	0	224
300— 600	39	68	68	54	19	5	0	253
600— 900	4	21	49	55	32·5	7	2	170·5
900—1200	2	9	12	24	25·5	13	2	87·5
1200—1500	0	1	10	16	18	7	1	53
1500—1800	0	0	5	7	11	10	1	34
1800—2100	1	1	2	12	7	7	0	30
Totals	142	165	184	186	118	51	6	852

Let there be two samples n_1, n_2: let $n_1 = p_1 + q_1$ where p_1 = number marked by some character (*e.g.* the fact of having three epidemics of plague); let $n_2 = p_2 + q_2$. The standard deviation of $p_1 = \sqrt{\frac{p_1 q_1}{n_1}}$ and of $p_2 = \sqrt{\frac{p_2 q_2}{n_2}}$. The percentage difference between the results of the two samples is $100\left(\frac{p_1}{n_1} - \frac{p_2}{n_2}\right)$ and the probable error of the difference is:

$$67{\cdot}449 \times \sqrt{\frac{p_1 q_1}{n_1^3} + \frac{p_2 q_2}{n_2^3}} \quad \text{.....(1).}$$

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TABLE XI.

Number of epidemics calculated for the selected villages with populations up to 2100, *on the basis of the rest of the district, attention being paid to size.*

Number of epidemics	Actual number of villages	Calculated number
0	13	18·52
1	16	24·61
2	27	31·23
3	35	34·19
4	30	24·95
5	23	12·10
6	3	1·40
	147	147

$P = 0{\cdot}006$.

We might compare the differences of percentages of villages infected 0, 1, 2, etc. times in each population group from the two sets of villages with the probable errors determined by (1).

This test is open to criticism. The use of the probable error based on the standard deviation as determined from the ordinary binomial requires for complete validity two assumptions, (1) that the events are really independent, (2) that the chances for and against the event are not very widely different. With regard to the latter objection, it can be met by only comparing groups in which not less than, say, 10 % of the villages were infected. The former objection cannot, however, be evaded in this way. It is likely that the fact of one village being infected is not independent of the presence of infection in another village. It must, however, be observed that if plague outbreaks are mainly attributable to recrudescence and not to importation, the condition would be more nearly fulfilled, although even in that event we cannot neglect entirely the factor of common meteorological or biological conditions. On the whole, it seemed just worth while applying the test to a few groups; this has been done and the results appear in the short Table XII. Although the differences are all less than three times their probable errors, they all exceed two and a half times this function so that it may justly be said the inferences to be drawn in no way conflict with those already stated.

Having reached the conclusion that proximity to a railway does increase the liability of a village to recurrent epidemics, I attempted to measure the intensity of the relationship. To do so we must employ

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the methods of multiple correlation, for we have not two variables but three, viz. (1) proximity to a railway, (2) size, (3) number of epidemics. We must determine the correlation between two of these variables for a constant value of the third, the coefficient of "partial" or "net" correlation[1].

TABLE XII.

Comparison of certain groups of villages.

Group: Population	Group: Epidemics	Percentage of such villages in the sample within 2 miles of the railway	Percentage of such villages among the villages more than 2 miles from the railway.	Difference ± probable error	Difference divided by its probable error
0—300	1	15·22	26·88	−11·66 ± 4·04	2·89
300—600	3	34·78	21·34	+13·44 ± 5·05	2·66
600—900	4	37·50	19·06	+18·44 ± 6·97	2·65

I first determined the ordinary coefficients of correlation and found:

(1) Between size of village and number of epidemics, $r = \cdot 594$.

(2) Between proximity to railway and size of village, $r = \cdot 154$.

(3) Between proximity to railways and number of epidemics, $r = \cdot 265$.

(1) was determined by the ordinary product moment method, (2) by a new process due to Pearson[2], (3) from a fourfold table[3], the divisions being for villages (*a*) those within two miles of the railway, (*b*) those more than two miles from the railway; for epidemics—(*a*) not more than two epidemics inclusive, (*b*) three or more epidemics. Finally I obtained the correlation between proximity to railways and number of epidemics for a constant size of village. This was found to be ·22. The question then arises as to the probable error of this coefficient. Were the partial correlation deduced from coefficients evaluated by the product moment method, it is known[4] that the ordinary formula can be used[5]. In this case, one at least of the total correlations is subject to a larger probable error than that based on product moments. I think, therefore, we should assume that the

[1] Yule, *Proc. Roy. Soc.* 1897, LX. 477.

[2] Pearson, *Biometrika*, 1909, VII. 96.

[3] Pearson, *Phil. Trans.* A, 1895, CXCV. 1.

[4] Yule, *Proc. Roy. Soc.* A, 1907, LXXIX. 182. Heron, *Biometrika*, 1910, VII. 411.

[5] $0{\cdot}67449 \times \frac{1-r^2}{\sqrt{n}}$.

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partial coefficient is subject to a probable error about three times the size of that given by the ordinary formula[1]. The formula gives in our case ·02, so that we can reasonably take the correlation between proximity to railways and number of epidemics as ·22 ± ·06. As the coefficient is more than three times its probable error, we may have some confidence that it indicates significant relationship between proximity to railways and number of villages.

One further point may be considered. Can we explain, on the basis of this work, the heterogeneous character of the epidemic distribution in Amritsar noticed in the first report? We can, I think, safely assert that this is one of the factors in operation, but certainly not the only one. I find on considering the villages exclusive of these within two miles of a railway, that the distribution is by no means a random one. This was to be expected, since even in regard to proximity to means of communication, the analysis here described is not exhaustive. Nearness to highways for wheeled traffic is very likely also a circumstance of importance, but, since we have evidently no satisfactory way of judging as to which roads marked in the maps are or are not greatly frequented, statistical analysis can hardly be applied to the subject.

The conclusion to be drawn from this study is therefore, that *the villages near a railway are subject to a higher rate of plague incidence than other villages and that this difference is not to be attributed to the smallness of our experience but may be considered an epidemiological fact.*

We must now inquire more particularly what is the epidemiological significance of the results obtained. Proximity to a line of railway might lead to an increased incidence of plague for all or any of the following reasons.

(1) Increased *personal* communication between villagers and centres of constant infection.

(2) Increased facility for the transit of merchandise, especially grain which might convey infected rats or possibly fleas.

(3) Some peculiarity either of the villages or the inhabitants of villages lying near the railway unconnected with (1) and (2). Since we have no extrinsic evidence pointing to condition (3), we are, for practical purposes, left to decide between (1) and (2). In a district containing large and important cities, cities of more than local fame, personal intercourse by railway is likely to be maximal, especially if any section of the

[1] This is a very generous allowance; in the particular case, I find, the probable error of the total coefficient (3) only 2·2 times the value deduced by the ordinary method.

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inhabitants owing to racial or religious peculiarities are given to travel. On the other hand in a district without large towns personal intercommunication between villages and such towns as exist will be much less, although the railway may be extensively used for the transit of goods. Amritsar fulfils the conditions requisite for much personal intercourse. Amritsar city is one of the largest and most important, if not the most important, cities in the Punjab. The district contains a considerable percentage of Sikhs who, as I understand, are extremely prone to travel and regard Amritsar city with religious veneration. On the other hand, Rohtak is an almost purely agricultural district. It contains no towns of more than local importance and practically no Sikh inhabitants. These remarks apply in the main to a third district, Gujrat, the data from which came into my hands since the results last communicated were worked out. From the information before me, I should infer that the manufacturing or commercial interests may be stronger in Gujrat than Rohtak but that from the point of view of personal intercourse it falls into line with that district. If, therefore, (1) be the true explanation, we ought to find that the partial correlation coefficient (constant population) is larger in the case of the data for Amritsar than for Rohtak or Gujrat.

Taking the same limits of size and adopting the same measure of accuracy as for Amritsar, I calculated the various constants for Rohtak and Gujrat, finally obtaining as partial coefficients of correlation between proximity to railways and frequency of epidemics, for a constant population:

$$\text{Rohtak, } \cdot 12 \pm \cdot 09,$$

$$\text{Gujrat, } -\cdot 18 \pm \cdot 06.$$

I have adopted the same generous margin, viz. three times the probable error given by the ordinary formula; allowing for this, we can hardly say that there is much evidence of any influence in the case of Rohtak. The coefficient for Gujrat is actually negative, pointing to some other influence, possibly a spread of infection from the margin of the district which obliterates any effect such as we have found in Amritsar.

These results amount, I think, almost to a proof of the correctness of hypothesis (1).

In our previous reports, Major Lamb and I devoted much attention to ascertaining whether the genesis of plague in the districts we investigated were in the main an affair of importation of infected

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material (or animals) or due to the recrudescence of a dormant process. As stated at the beginning of this report, were the former hypothesis to express a truth, we should expect villages near an important means of communication to suffer with excessive frequency from epidemics of plague. If on the other hand, an epidemic is generally due to recrudescence, it is difficult to see why proximity to a railway should be of any importance unless we adopt (3) *supra*, which, as previously remarked, has nothing in its favour.

But not only do we conclude generally in favour of the importation hypothesis in the case of Amritsar, further we have evidence as to the conditions under which it will be valid. A comparison of the three districts seems to make it highly probable that it is importation by persons or personal effects which determines the spread of epidemics, not so much the mercantile goods' traffic.

While fully admitting the imperfections of our material from the statistical point of view, I think the conclusions just stated must be regarded as at least highly probable. It is, however, quite clear that the factor here demonstrated is not the only nor perhaps the chief one at work. This is shown by the absolutely small value of the coefficient even for Amritsar district, where the conditions are most favourable, and its total extinction in the agricultural districts of Rohtak and Gujrat.

Conclusions.

1. *In districts containing large cities, villages near a line of railway are liable to a higher rate of plague epidemics than villages not so situated.*

2. *This is probably due to increased opportunities for personal transit, not to the exportation or importation of merchandise.*

3. *In a district favourably situated for personal intercommunication, the spread of plague can be much better explained on the hypothesis of reimportation than on that of recrudescence.*

4. *In purely or mainly agricultural districts, proximity to railways does not increase the liability to plague outbreaks.*

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APPENDIX.

Further note on the actual and calculated frequencies of recurrent epidemics of plague.

In my former report (Vol. x. pp. 425–7 and Table XXIX) a special comparison was instituted between the actual frequencies of villages affected in successive years and the numbers calculated on the assumption that plague in one year did not predispose to an epidemic in the same village in the following year. In testing agreement between expectation and observation, I adopted the process used in earlier sections of the report. Now, while the value of χ^2 is evidently some measure of agreement in all cases, the value of P given by such a table as XXIX (*op. cit.*) cannot be compared strictly with the results obtained from the other tables since the whole of the chance distribution was not used. As the test presupposes a complete distribution, I have extended the comparison to the whole of the possible categories and we obtain the accompanying Table XIII. In this table the figures enclosed in brackets are the calculated frequencies on the hypothesis of independent chances. We shall obtain the same total frequency for calculated and observed values by grouping the results in several ways.

(1) If we consider each vertical column of the table as a distribution, we find the agreement to be measured by the values of P entered at the foot of each column.

(2) If we consider each sequence of two years separately and take a separative subdivision for each group of villages, giving 20 subdivisions in all, we have:

1903–4, $P = 0{\cdot}86$; 1904–5, $P = 0{\cdot}99$; 1905–6, $P = 0{\cdot}70$; 1906–7, $P = {\cdot}049$.

(3) If we group all the villages together, irrespective of size, for each sequence, leaving only four groups, the values of P become 0·02, 0·78, 0·04, 0·39.

Of these arrangements (1) is perhaps to be preferred.

As will be seen from the general trend of the values, this more detailed comparison leads to precisely the same conclusion as that given in the text. In other words, the difference between observed and expected values in this series is not sufficient to warrant a belief that recurrences occur with undue frequency. The conclusions based upon this finding in the first report may therefore stand.

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TABLE XIII.

Recurrence of plague in villages: Amritsar district.

The figures are numbers of villages, those in brackets being the numbers calculated on the supposition that the distribution of plague in one year is independent of the preceding and succeeding epidemic distribution.

Year	Population groups				
	400—500	500—700	800—1000	1000—1200	1200—1400
1903 not 1904	32 (34·35)	36 (44·73)	29 (32·07)	16 (17·97)	9 (10·35)
1904 not 1903	12 (14·35)	28 (36·73)	14 (17·07)	12 (13·97)	7 (8·35)
1903 & 1904	17 (14·65)	39 (30·27)	32 (28·93)	27 (25·03)	25 (23·65)
Neither year	36 (33·65)	63 (54·27)	22 (18·93)	12 (10·03)	5 (3·65)
1904 not 1905	14 (12·56)	22 (24·62)	4 (6·64)	6 (8·15)	5 (4·17)
1905 not 1904	40 (38·56)	60 (62·62)	41 (43·64)	20 (22·15)	13 (12·17)
1904 & 1905	15 (16·44)	45 (42·38)	42 (39·36)	33 (30·85)	27 (27·83)
Neither year	28 (29·44)	39 (36·38)	10 (7·36)	8 (5·85)	1 (1·83)
1905 not 1906	38 (39·12)	85 (87·29)	52 (55·62)	25 (28·48)	23 (25·22)
1906 not 1905	11 (12·12)	8 (10·29)	1 (4·62)	3 (6·48)	0 (2·22)
1905 & 1906	17 (15·88)	20 (17·71)	31 (27·38)	28 (24·52)	17 (14·78)
Neither year	31 (29·88)	53 (50·71)	13 (9·38)	11 (7·52)	6 (3·78)
1906 not 1907	15 (13·86)	8 (15·01)	4 (8·58)	8 (8·79)	7 (4·07)
1907 not 1906	36 (34·86)	57 (64·01)	43 (47·58)	25 (25·79)	25 (22·07)
1906 & 1907	13 (14·14)	20 (12·99)	28 (23·42)	23 (22·21)	10 (12·93)
Neither year	33 (34·14)	81 (73·99)	22 (17·42)	11 (10·21)	4 (6·93)
	$P=0·99$	$P=0·27$	$P=0·53$	$P=0·95$	$P=0·80$

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XLV. STATISTICAL INVESTIGATION OF PLAGUE IN THE PUNJAB. THIRD REPORT: ON SOME OF THE FACTORS WHICH INFLUENCE THE PREVALENCE OF PLAGUE.

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With Map and 5 Figures.

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I. *Introduction.*

In a first report, written in conjunction with Major Lamb[1], evidence was presented which made it seem probable that the recurrence of plague in the villages of the Punjab and United Provinces, which had been specially investigated, could in the main be attributed to reimportation of the disease; such a mode of origin appeared epidemiologically of greater significance than a possible awakening of

[1] "On the spread of epidemic plague through districts with scattered villages." *Journ. Hyg.* x. (1910), p. 350.

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some *materies morbi* which had remained dormant since an earlier visitation. We also pointed out that the proportion of small villages which remained free from plague in any epidemic was much higher than that of large villages or towns (see especially Tables LIV–LIX, pp. 659–661, and I. p. 676). It was further shown that, apart from questions of size, the distribution of infected villages, particularly in the Amritsar district, suggested that certain regions might be peculiarly exposed to infection. The suggestion was made that proximity to habitual lines of travel was a factor in this result, railways being specially mentioned. In a second report (p. 47 *supra*) I have shown that, in Amritsar district, proximity to the railway has undoubtedly influenced the distribution of infected villages. At the same time, however, it was pointed out that the result of a similar inquiry in the case of another district, Gujrat, indicated that proximity to railways could not be the sole nor even the chief factor in disseminating plague.

In the two previous reports, attention was almost entirely confined to the simple fact of infection or non-infection, a division into two mutually exclusive classes being all that was attempted. Little or no heed was paid to the remarkable differences in rates of mortality among infected villages, which are nevertheless sufficiently marked in character. In order to give the reader of this report some idea of such differences, Tables I–VIII have been prepared; they are mere random samples intended as illustrations of a host of similar facts. These and kindred observations led me to formulate an epidemiological problem in the following terms—What circumstances determine the *extent* of an epidemic when plague has once manifested itself, why is it that such great discrepancies are observable in the rates of mortality? The present report is a systematic attempt to analyse the more prominent of the factors which could be thought responsible for the results. While it will undoubtedly be found that much remains obscure, I am hopeful that some light has been thrown upon points not without epidemiological importance.

II. *Nature of the statistical materials and possible sources of error and uncertainty.*

The districts under observation are Gujrat, Hoshiapur, Rohtak and Amritsar in the Punjab, and Mozuffarnargar in the United Provinces. A few general particulars relating to these districts have been collected in Tables IX and X. It will be noticed that Amritsar is the only one

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in which agriculture does not employ at least half the inhabitants and also the only one possessing a considerable urban population. The returns of plague-deaths from the numerous villages within these districts were made under the personal supervision of the late Major Lamb and numerous corrections on the data sheets together with the correspondence exchanged between Major Lamb and the local officials testify to the care with which the figures have been scrutinised. As has been frequently pointed out, however, some degree of uncertainty must always exist. Thus, when the prevalence of plague is low, there is perhaps a tendency to report deaths due to plague under other headings; conversely when the disease is widespread, deaths not caused by plague may possibly be credited to this disease. Naturally the magnitude of this error cannot be determined from internal evidence[1], comparable statistics of deaths from all causes are not at hand, but its possible existence must not be ignored. A second source of error is the fact that the population returns are based on the census of 1901, *i.e.* they refer to a date several years earlier than most of the epidemics in our series. If we could be certain that all the villages have increased or decreased in the same ratio, percentages calculated on the 1901 figures while absolutely wrong would still be comparable *inter se.* Such an assumption would, however, require strong external evidence to justify it and we may be fairly confident that the (compulsory) adoption of a 1901 population basis has introduced a somewhat considerable source of error into our discussion of mortality rates. A third source of error, or rather, to speak more correctly, of uncertainty, is due to the impossibility of checking the returns of deaths in our data schedules against those which figure in the weekly lists issued by the Government of India. The summed totals from the Commission's data are widely at variance with those given in these weekly lists. It would seem, however, that the difference is not caused by any serious error in either set of figures, since there is a tolerably close agreement between both records with regard to specific towns. The most plausible suggestion is that there has been some different delimitation of the districts for the purposes of the two series. One remark may be made with regard to the weekly returns; little or no weight can be attached to case mortality deduced from a comparison of the reported seizures with the reported deaths for the same or any previous week. In many

[1] "From a careful study of deaths from all causes contained in the death registers we have arrived at the conclusion that very few deaths from plague escape notification." ("Plague in Belgaum," *Journ. of Hyg.* x. 1910, p. 455.)

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districts such a comparison would lead (has indeed led certain newspaper writers) to the conclusion that the average case mortality is well over 90 %, an improbable figure for bubonic plague.

It will hardly be questioned that the material upon which my analysis depends is subject to very considerable errors. These errors reflect in no way upon those who have spent so much time and care upon collecting and revising the data; indeed it may safely be asserted that the present material has attained a higher standard of precision than any previous records at all commensurate with it in scope. Nevertheless, the possible sources of error are so important that statistical deductions can only be made with considerable hesitation. When the whole or most of the evidence points consistently in any one direction, we may justly have some confidence that the conclusion indicated is sound; but we must neither push our inferences too far, nor expect very detailed results. It is for these reasons that I have often presented the same material tabulated with what may seem unnecessary repetition and that I have drawn conclusions rather cautiously, even when the results of tabulation or analysis seem to force them upon one's notice. I have also in most cases omitted such subdivisions of data as would fairly involve the consideration of the so-called "errors of random sampling." I shall, indeed, from time to time refer to certain statistical questions of this nature, but in most cases such applications would only tend to clothe the results in a garment of spurious precision. In this connection I may say that tabulation has often been carried to decimal places beyond the limits of material accuracy. This has been done partly in the interests of neat statement, but more to avoid the illogical but almost instinctive conclusion on the reader's part that results given in round numbers are based on careless or approximate methods of calculation. I have thought it well to emphasize this question of "material errors" at the outset; if the subject is not again discussed in detail, I hope the reader will not imagine it to have been lost sight of and that, on the other hand, doubt or hesitation will not be attributed to pedantry. I have used my best efforts to place just as much weight upon my statistical foundations as they will bear and no more.

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III. *The influence of length of exposure to infection upon total mortality.*

It has already been stated that previous work inclines one to believe that the origin of most village outbreaks is the importation from elsewhere of the disease—how this takes place will be matter for subsequent consideration. It will at once seem probable that a village which has become infected early in the season must show a greater number of cases in the whole epidemic than one not "exposed to risk" until later in the plague season, which extends in these districts roughly from November to July. Hence it might be supposed that the differences in rate of mortality as exhibited in the annual figures, which were the starting point of the present inquiry, depended merely upon this circumstance. Tables XI–XVIII contain a first study of the influence of length of exposure to infection upon total mortality; they were prepared in the following way.

All the Hoshiapur villages without exception, and all villages of less than 10,000 inhabitants in the four other districts, were grouped according to the month of first infection. That is to say, a list was made of villages which first reported cases in—*e.g.*—March, their total population, total numbers of deaths, percentage mortality, etc. were noted and the same process was applied to every other month in the plague season. The very few[1] villages which reported cases as early as October or November were grouped with December infections and the still fewer villages which escaped until August either omitted or reckoned in the July total. If a village had a free interval of three months or more—*e.g.* if it returned cases in January and no more until May—it was reckoned twice, once under each month. The salient features of these tables are indicated in the following notes.

Amritsar. 1902–3. The February infections show a higher average mortality than those of January.

1903–4. A steady decline from February. The first two months are based upon statistically inadequate returns.

1904–5. A similar picture to that of 1903.

1905–6. A practically uniform decline; there were only two infections before January.

[1] For the epidemics and districts where these conditions fail a separate table (XVIII A) has been prepared.

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1906–7. Villages first infected in January, February or March differ little in rates of total mortality or average populations.

Hoshiapur. 1901–2. February and January rates similar but the mean population of the January villages markedly higher.

1903–4. February rates markedly higher than those of previous or subsequent months. (Let me repeat that what I call *e.g.* the February rate means the total rate *for the whole epidemic* of villages first infected in February, *not* the number of deaths reported in that month alone.)

1904–5. No great diminution in the rate before May, but villages first infected in December show the greatest rate.

1906–7. The marked fall does not begin until April. February worse than January.

Gujrat. 1903–4. Very irregular. The first three months based on too small figures.

1904–5. A fairly regular fall from the beginning but the March and April rates are sensibly equal.

1906–7. February once again predominant, but the earlier months depend on small figures.

Mozaffarnagar. 1903–4. Figures too small to be reliable.

1904–5. Shows a tolerably smooth fall.

1905–6. Also unreliable numbers.

1906–7. A maximum in March.

Rohtak. 1904–5. December below any month before April.

1906–7. February and March about equal. December and January unreliable.

The general conclusion suggested by a study of these tables would be that, on the whole, the earlier the infection is introduced the greater the resultant mortality but that the relation is neither very close nor straightforward. Let us now scrutinise the figures more closely. As will have been noticed in the tables, the average population of villages infected early in the season is generally much greater than that of villages having a longer free interval (a circumstance which might have been predicted from the evidence contained in earlier reports). It is therefore necessary to isolate the influence of earliness of infection upon total mortality from that of size; this has been attempted in two ways. Before discussing these methods, however, the reader must be clear as to exactly what problem we are now considering.

We are at present solely occupied with the influence of length of exposure upon total mortality. The plague season extends for all practical purposes when we deal with villages, not large towns, from

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November to July; hence a village reporting cases in December has before it eight months during which cases might occur; if the infection does not reach the village until May then only three months can yield cases. It does not, of course, follow that these possibilities are realised, the force of the infection may spend itself in one or two months; so to speak, the danger is potential and need not become actual; this distinction will be examined later on. If now we take the number of months from the date of first infection to the end of the season as one variable and the total recorded deaths as the other variable, the correlation between the two will be a measure of the relationship between total mortality and length of possible exposure. If for number of deaths we substitute population, the correlation will measure the relation between size of village and length of exposure. It must be carefully noted that the former of these correlations does not measure the relation between any meteorological condition and the number of plague deaths. Such influences require and will receive separate consideration, but they cannot profitably be discussed until the factor of mere duration in time has been examined and measured. I now return to the methods which have been employed to study this problem.

(1) Groups of villages having nearly the same population were formed and the correlation between number of months of exposure and total deaths was determined by the direct product moment method without grouping. By this process the population factor was experimentally kept constant, but only very roughly, as will be seen.

(2) All villages of not more than 3000 inhabitants were tabulated, together with their plague histories, and the following coefficients evaluated, by the product moment method.

(i) Absolute population and number of cases.

(ii) Absolute population and length of possible exposure.

(iii) Length of possible exposure and total number of deaths from plague.

We have, if r_{pd} = correlation between absolute population and length of exposure, r_{pm} = the correlation between absolute population and absolute number of deaths and r_{dm} = the correlation between absolute number of deaths and length of possible exposure,

$$\frac{r_{dm} - r_{pd} \times r_{pm}}{\sqrt{1 - r^2_{pd}} \times \sqrt{1 - r^2_{pm}}}$$

for the correlation between length of possible exposure and number of deaths for a constant population.

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The results of both methods appear in Tables XIX–XXI[1]. Scientifically the second process is to be preferred because it was impossible to obtain a reasonably large group of villages affected in any one epidemic which were sensibly equal in populations. It is not, therefore, surprising that the coefficients in Table XIX are very variable although of the same order of magnitude as those in Table XXI. With special reference to the values of Table XXI it will be noticed that all epidemics in Hoshiapur give results in good agreement one with the other. One Gujrat coefficient is large, one Amritsar and one Mozuffarnargar value seem unduly low; in the latter case the arithmetical work has been repeated without the discovery of any slip in computation.

The question then arose whether the relatively small sizes of the coefficients were due to any marked departure from linearity so that the coefficient ceased to be a satisfactory measure of inter-relationship when taken by itself. It is hardly possible, in the present state of knowledge, to consider the partial regression coefficients directly from this point of view; sufficient information for our purpose can, however, I think, be obtained from a study of the total regression of deaths upon length of exposure. The mean numbers of deaths for each length of exposure were plotted from each set of data. The resulting points were not collinear but it was noticed that the most divergent means were based on few observations. In the case of Hoshiapur 1906–7 the nature of the regression was completely studied. In this case r was ·281, η (the correlation ratio[2]) ·287 and the ratio of $\eta^2 - r^2$ to its probable error[3] 1·34. In other words the regression was effectively linear. The diagram (Fig. 1) shows the mean points and the best straight line; it should be noted that in fitting the line each point is weighted with the number of observations on which it is based, so that the diagram exaggerates the divergence from linearity. I also found that the omission of the scattered observations derived from the early months of the epidemic did not, in the cases tested, lead to very substantial alterations in the coefficients although their values were increased. It does not, therefore, seem probable that a close relationship between length of possible exposure and total mortality has been masked by some peculiarity in the regression curve.

[1] Space does not permit of publishing all the correlation tables; as an illustration, the set relating to Amritsar 1902–3 is given (Tables XXI A, XXI B, XXI C).

[2] Pearson, *On the General Theory of Skew Correlation and Non-Linear Regression*, London, 1905.

[3] Blakeman, "On Tests for Linearity of Regression in Frequency Distributions," *Biometrika*, IV. (1905), p. 332.

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I next considered the relation between possible and actual exposure, *i.e.* I ascertained whether those villages which, owing to early importation of disease, might have been infected in the greatest number of months actually were so. Table XXII shows the average durations of plague in villages first infected in each month in sequence, for two epidemics which attacked a sufficiently large number of villages to render mean values significant. Naturally the actual is less than the possible exposure but varies fairly uniformly with the latter (Fig. 2). I next attempted to measure the influence of date of first infection upon total mortality, *i.e.* I sought to discover if more cases resulted from infection n—*e.g.*—months 1 and 2 than from infection in months 2 and 3. In

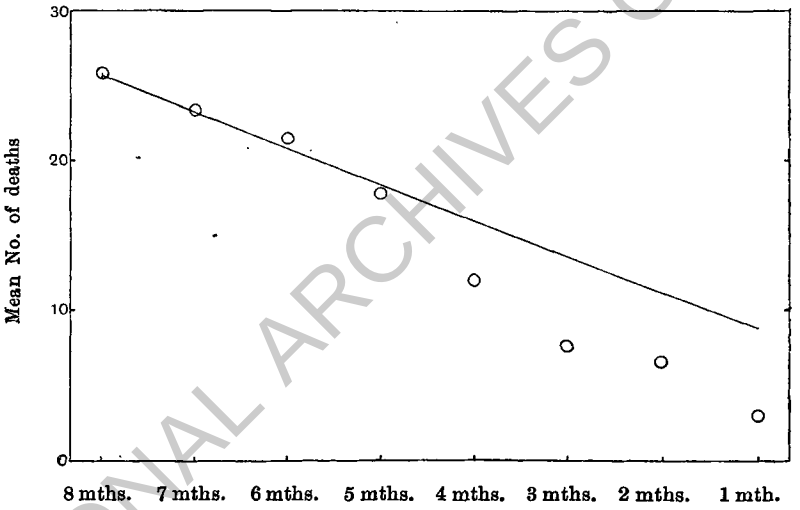

Fig. 1. Hoshiarpur, 1907. Mean no. of deaths for villages exposed during different periods. (The line is $y = \cdot 24\,x$ where y is measured from the mean no. of deaths, 19·1, and x from 5·25 months. The units are 10 deaths and 1 month.)

Gujrat for the epidemic of 1906–7 (villages of populations up to 3000) villages which reported deaths in two months only were tabulated, the three coefficients of correlation were calculated and the value of the relation between date of first exposure and number of deaths for a constant population proved to be $\cdot 26 \pm \cdot 04$. It would therefore seem that given an equally long effective exposure there is a slight tendency for more deaths to occur early in the season than later on. All these

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results tend very clearly to show that two factors influence the death rate, (*a*) length of possible and actual exposure, (*b*) *date* of first exposure. They show not less clearly that neither of these factors nor both combined suffice to explain the wide discrepancies in death rates which were the starting point of the inquiry.

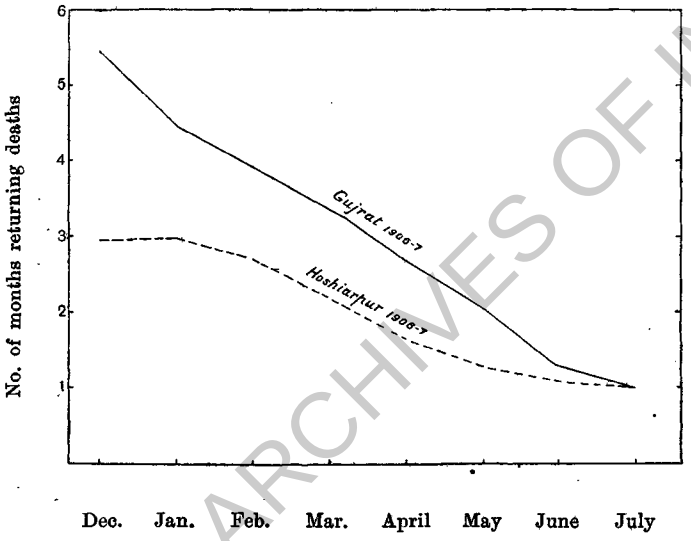

Fig. 2. Month of first infection.

The conclusions of this section may be summarised in the following way and, in view of the reasonable uniformity in analytical results, I have fair confidence that they correctly represent the epidemiological facts.

(1) *Other things being equal,* the rate of mortality varies with the length of possible and actual exposure but the relation is not close.

(2) Given the same period of actual exposure, more deaths will result from infections early in the epidemic season.

(3) Large villages tend to be infected early in the season, but the relation between size and date of infection is not close.

(4) The bulk of the difference between rates of mortality in individual villages of the same size cannot be explained completely by these results.

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IV. *The relation between absolute size and relative mortality in villages.*

In Tables XXIII and XXIV, the data are so presented as to allow of a comparison between the rates of plague mortality in large and small villages. Table XXV gives the urban data while Tables XXVI—XXXII permit of a more detailed analysis of the village returns.

The general trend of these figures points to the conclusion that small villages are usually subject to a heavier rate of mortality, *when infected*, than larger ones. In certain cases, especially perhaps Gujrat, the difference is very considerable; in one instance, Rohtak, the rates go in the opposite direction. As will appear, this exception can be partly referred to the local differences which are found in Rohtak in respect of incidence, but even making this allowance, the Rohtak returns must be considered to form an exception from the present standpoint (Table XXXII)[1].

Before we attempt to explain these differences it may be asked whether they can possibly be referred to errors of random sampling and a few words may be devoted to this point.

If we wish to determine the probable errors of mortality rates for plague the usual method, founded on the binomial standard deviation, is inapplicable since the condition that the events are independent one of another is not fulfilled[2]. The following modification of the usual process is perhaps worthy of adoption in such instances. We will take a specific example.

In Gujrat, during 1906–7, considering only villages which first reported deaths in March, the following results were obtained:

Villages of Populations 1000—2000.

Total population	Total deaths	Death-rate per cent.
36461	3983	10·92

Villages of Populations less than 700.

38522	5779	15·0

Let us calculate the error of sampling in this case.

The weekly official returns enable us to determine the number of deaths in each month throughout the epidemic, but since the total

[1] See also Tables LXV and LXVI.

[2] See Yule, "An Introduction to the Theory of Statistics," London, 1911, Chap. XIV; Greenwood and Candy, *Journ. Roy. Statistic. Soc.* LXXIV. 1911, pp. 375–6; Yule, *ibid.* 398–9.

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deaths recorded in the data prepared for Major Lamb do not correspond exactly with the weekly figures (*vide supra*) the latter were reduced to the same dimensions as the former, by multiplying throughout by

$$\frac{\text{Total Deaths in Commission's Data}}{\text{Total Deaths in the Weekly Returns}}.$$

In the end, the following table was obtained:

Month	Population at risk	Chance of a plague death. Assuming independence of chances
March	234,017	·0221
April	413,096	·0247
May	505,874	·0436
June	509,820	·0199
July	503,481	·0029

We might consider each month's "exposed to risk" as a separate "population" and the chances of each person dying of plague as independent and constant *for that month.* Then, if the most probable number of deaths in any month be m, and the binomial standard deviation for the same month σ_m, the total number of deaths to be expected throughout the season will be $S.\ m$ with a standard deviation of $\sqrt{S\sigma^2_m}$. The calculation was carried out as shown in the next table:

A. *Villages of Population* 1000—2000.

Month	Expected deaths	Population at risk	Square of standard deviation
March	804	36,461	786·26
April	882	35,657	860·18
May	1517	34,775	1450·82
June	662	33,258	648·83
July	96	32,596	95·72
August	—	32,500	—
	3961		3841·81
			S.D. 61·98. Prob. error 41·8

B. *Villages of Population* 0—700.

March	850	38,522	831·25
April	932	37,672	908·94
May	1603	36,740	1533·07
June	699	35,137	685·09
July	101	34,438	100·70
August	—	34,337	—
	4185		4059·05
			S.D. 63·71. Prob. error 42·97

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Finally, we reach:

Population-group	Calculated number of deaths	Actually observed number of deaths	Difference between the actual and calculated numbers divided by the latter's "probable error"
1000—2000	3961	3983	·53
0—700	4185	5779	37·1

If such a method of testing be admitted (it is at best a rough and ready process to be used with caution) the difference between the rates of mortality in the example can hardly be due to errors of random sampling.

The question of how to calculate probable errors in material of this class has a certain theoretical importance; practically, in the case before us, I do not attach any great value to the inquiry. We are only interested in large differences, and it may be doubted whether errors of random sampling are of much importance in comparison with the material errors of record mentioned above (p. 64). The numbers are large and the reader should examine the evidence as a whole, particularly Tables XXIII—XXXII. He will then, I believe, reach the conclusion that, on the average, in most epidemics the relative mortality from plague in small villages actually exposed is greater than upon large ones, the conclusion stated a few pages back. It must be remarked again that the proportion of villages which *escape altogether* is larger when we deal with the smaller communities (see Tables XXXIII, XXXIII A, and also this *Journal*, Vol. x. pp. 351, etc.). Table XXXIV illustrates the difference in the percentage rates which is effected by the inclusion not only of villages actually infected but also of those which might have been[1]. It will be noticed that in some, but not all, cases this inclusion has sufficed to turn the scale in the other direction. On the whole, then, a review of the somewhat considerable mass of data here collected seems to prove that, given an equal period of exposure to infection, small villages suffer more severely from plague than large ones, and we have no ground for attributing this result to errors of sampling. Can they be due to errors of record? Evidently a mistake in the returns from a small village will lead to a relatively greater error in the mortality rate than an inaccuracy of the same absolute magnitude in a village of larger size. But it would be futile to seek an explanation of our result in this way. We should have to suppose that errors of record consist in systematic over-statements of deaths in small villages and equally systematic under-statements from large villages. Such an assumption would be quite

[1] See also Tables LXV and LXVI.

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arbitrary without positive evidence and may be dismissed. I see no reason to attribute the result to errors of record.

We must therefore attempt to interpret these curious and interesting facts but, before doing so, it is well to ask whether the same or similar circumstances have been noticed before. Hankin[1], in 1905, pointed out a differential mortality rate in communities of different sizes; he contrasted village mortality with that of certain towns in Bombay. Hankin also called attention to the probable occurrence of a similar phenomenon in the Black Death. In Appendix I, I deal with such statistics as I could obtain bearing on the historic plagues; it will be found that these, so far as they go—unfortunately not very far—bear out Hankin's suggestion. They lend some support to a belief that we here have to deal with a general epidemiological phenomenon. It is to be noted that Hankin's work and my appendix are chiefly occupied with the contrast between urban and village mortality. In the case under discussion, the difference is found to be carried further and still to prevail amongst communities of the same general type.

This distinction is of epidemiological importance since the difference between a town and a village is not merely one of population, so that variation in rates of mortality might well depend upon factors which can hardly operate in village communities. In what follows I shall attend only to the latter class.

To throw light upon our problem we need first to consider how plague arises in a village and then how it spreads; let us examine these points in order.

I do not question that human epidemic plague (of bubonic type) in villages is derived from a rat epizootic by the mediation of rat fleas, particularly *X. cheopis*. Further, the evidence presented by Major Lamb and me (*op. cit.*), appeared to find its best interpretation on the hypothesis that village infections do not arise by recrudescence but from the introduction of infective material from outside. It must also be regarded as probable that neither transference by human contagion nor even by human parasites is *epidemiologically* important for the spread of village plague in the Punjab.

We shall, therefore, be justified in laying it down, as a fair working hypothesis supported by direct and indirect evidence, that in the great majority of the villages now being discussed epidemics have arisen in the following way:

[1] *Journ. Hyg.* v. (1905), p. 56.

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At some point, or points, within the village an "effective" centre of rat plague has been established which then acts as a place of distribution for rat fleas some of which are infective. By "centre of effective occupation," I mean a place in which the rats *inhabiting* the house or hut have become infected and I distinguish an importation which has succeeded to this extent from the mere casual introduction of *materies morbi* not followed by an epizootic.

There are at least four ways in which an "effective" centre may arise:

(1) By the introduction of an infected human being.

(2) By the human importation, on the person or in the clothing, of infective fleas.

(3) By the importation of infective fleas or infected rats in merchandise.

(4) By the direct immigration of infected rats.

The last, having been specially considered by the Commission and found to have no apparent importance[1], need not detain us. With regard to the other methods[2], plainly, certain villages, owing to their geographical situation or some local peculiarity, might be specially prone to receive infection; but such factors have no necessary relation to size. The process initiated in the neighbourhood of an "effective" centre might be compared to a battery discharging shots—infective fleas—at random, the range being limited to a single house or group of dwellings and the rate of discharge increasing up to a certain limit. Given the establishment of one or more such primary "effective" centres, the question arises as to how the epidemic is spread through the community.

All the methods by which a centre is established *ab extra*, will still apply for the establishment of secondary centres within the community, and in addition we have to reckon with chances of spreading through the rat population by the transference of infective fleas from individual to individual. On the latter hypothesis, we should, perhaps, expect to be able to trace the lines of spread throughout the village starting from the initial centres and to find the infected areas, both epidemic and epizootic, steadily widening from week to week around these foci, until finally the distribution becomes approximately uniform.

[1] *Journ. Hyg.* Plague No., VII. 1907, p. 907.

[2] I have purposely omitted certain entomological possibilities and considerations which are of moment but not, I think, in connection with the special topic discussed in this section.

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An examination of the weekly spot-maps for Belgaum[1] does not entirely justify such anticipations; it is impossible to trace in them any regular process of extension from initial centres throughout the community although there is evidence of a restricted area of dissemination around each focus[2]. The statistical evidence is equally inconclusive. Thus if plague tends to be uniformly spread through a village from the primary "effective" centres, we ought to find that the longer an outbreak endures the more nearly should the rates of mortality in different villages approximate, unless we assume that some factors correlated with absolute size arrest the extension. In other words, we ought to find that the difference between the rates of mortality in large and small villages should be greater in those only returning deaths in a single month than in villages affected in two or more months. I investigated this point, which was suggested by my colleague Dr Henderson Smith, in the way shown in Tables XXXV—XXXVIII. It cannot be said that these tables lend any real support to the idea that the difference in rates will diminish as we extend the duration of the epidemic. It cannot, of course, fairly be concluded from these results that the spread of an epidemic is not a consequence of a disseminated epizootic. The patchy character of the distribution may perhaps be accounted for in the following way. Let us suppose that at *a* and *b*, two widely separated spots, "effective" centres have been established. At and immediately around *a* and *b* there will be a considerable mortality among the rat population. This thinning of the ranks may induce an immigration of other rats so that the density of the rat population in any other places and therefore in the region between *a* and *b* will tend to be diminished. Some of the new-comers will be infected and, in course of time, the infected areas will extend, but the plague season may not last long enough for any distinct evidence of this to appear[3].

In the meantime, new centres may be established at a distance from *a* and *b* by the casual emigrations of infected rats from the original points or owing to chance intercourse of the rats from *a* and *b* with individuals domiciled elsewhere.

Interesting as this problem may be, its solution is not germane to

[1] *Journ. Hyg.* x. (1910), pp. 446, etc.

[2] Cf. Brownlee, *Proc. Roy. Soc. Edinb.* xxxi. (1911), p. 262.

[3] It is to be remarked that the daily movements of human beings about a village tends to vitiate any attempt to trace the course of dissemination from maps showing the dwelling houses of infected persons: see this *Journal*, Vol. vii. (1907), pp. 733, 860.

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the present inquiry. What *is* material, however, seems to be the apparent fact that the extent of an epidemic must largely depend upon the number of "effective" centres established early in the epidemic. The diffusive power of plague is evidently not so great that one "effective" centre is sufficient to develop a wide-spread outbreak. A sun-dried expanse of brush-wood may be completely consumed if a fire is kindled at a single point within it, but, in moister circumstances, many independent conflagrations will be required. The latter half of the simile appears to correspond to the case of plague.

On the whole we may lay it down as a working hypothesis that the extent of an epidemic will be determined in the first place by the number of independent "effective" centres established, mainly by importation, at a relatively early stage in the plague season. The degree of effectiveness of these centres will further depend on the density of the surrounding population. Now, if we return to the enumeration of possible manners in which the primary centres may be established, it seems clear *a priori*, that, as a general rule, the number of centres will increase steadily with the population. Again, if the population density be greater in large than in small villages, the efficiency of the centres should increase with the population.

An associated factor would also seem to favour an increased rate of mortality in large villages. The nearer two "effective" centres are one to another the more favourable the conditions for producing plague, since the population of the area lying between the two centres will be, if the latter are not far apart, exposed to two fires. The average distance apart of a series of balls thrown at random upon a surface of known area will depend on the number of balls and the size of the area within which they must fall[1]; consequently, if the area does not increase at the same rate as the number of centres, this circumstance would favour a relative increase of mortality in large villages. These *a priori* speculations lead to a result very different from the actual one. On the other hand, some considerations would point to a state of affairs qualitatively resembling what we do find.

Suppose the distribution of plague deaths *purely* random. Let the chance of a person dying from plague be p and of not dying q where

[1] I think, the average distance apart will vary as $\sqrt{\frac{A}{N}}$, where A is the number of units of area and N the number of imported foci of plague, but problems of this kind are intricate and the immediate practical importance of the matter hardly justifies a prolonged investigation.

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$p+q=1$. Then the proportional frequency of villages, each containing n inhabitants, which will report 0, 1, 2, etc., deaths is given by the terms of the expansion of $(p+q)^n$. Consequently the relative frequency of non-infected villages will be greater when n is small and the rate of mortality among the infected villages higher. The subjoined figures illustrate this

No. of deaths (to nearest unit)	Binomials $1000(\cdot01+\cdot99)^{100}$	Binomials $1000(\cdot01+\cdot99)^{500}$
0	366	49
1	370	149
2	185	224
3	61	225
4	15	169
5	3	101
6	—	50
7	—	21
8	—	8
9	—	3
10	—	1
% non-infected villages ...	37·0	4·9
% deaths *in infected villages*	1·58	1·051

If, however, we consider any of the tables, *e.g.* Amritsar figures in Table XXXIV, it will be found that while p and q are of the order ·03 and ·97, n is of the order 460 and 1540 in the two groups. Thus we see that the proportion of non-infected villages in each group and the difference in rate of mortality between the groups transcend any possibility of simple explanation along these lines[1]. We are accordingly forced to the conclusion that the observed results require the intervention of some other factor.

Let us return to modify our conception of the way in which the spread of plague through villages can be influenced. One fairly obvious modification is with respect to the density-factor. There must, one would suppose, be an upper limit to the degree of possible overcrowding and, although we might not expect it to have been reached in villages of the size contemplated here, we may suspect that such a circumstance has operated against the uniform increase of mortality rate with population. Perhaps the density of population increases with absolute size at a steadily diminishing rate, so that we might represent the change by some expression of the form $y=f(x)$, where y is the

[1] These remarks were suggested to me by Mr G. Udny Yule to whom I am further indebted for much valuable criticism of the first draft of this chapter.

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density and x the gross population, the function being such that $y = k$ is an asymptote. But in order to account for the diminution in relative mortality as absolute size increases, it seems further necessary to suppose that (*a*) the number of initial centres does not increase at the same rate as the population, if we assume that the extent of an epidemic is determined by the number of such centres, *or*, (*b*), that, with an increase in population, factors limiting the epizootic extension gradually come into play. In order to decide between (*a*) and (*b*) we should have to ascertain from a large sample of villages (1) the density of population, and (2) the number of "effective" centres in the early weeks of the epizootic in each village. We have naturally no data on this scale of minuteness; some particulars can, however, be learned from the study of two Punjab villages, Dhand and Kasel.

The particulars which interest us are as follows:

Village	Population	Density per acre	Persons per house	Persons per room*	No. of houses plague-infected in 1st week of epizootic†
Dhand	1920	70	4·6	1·9	4
Kasel	3938	100	4·9	2·5	6†

* Calculated on the assumption that houses with more than two rooms have on the average 3·5 rooms each.

† Inclusive of a house with a human case but no plague-rats.

The information is of course defective from our point of view. It cannot, for instance, be said that the presence of human cases or even plague-rats in huts early in the epizootic, constitute the houses necessarily effective centres in my sense. Again, the measure of density employed is open to criticism. Still, *prima facie*, it would seem that the change both in density and number of initial foci does not keep pace with the absolute increase of the population. It would not, of course, be difficult to construct formulae which give changes in mortality rate with increase of population comparable to the observed results. We need merely suppose that the density increases at a diminishing rate and that the number of centres increases absolutely but not relatively to the growth of population. An illustration of the sort of graphical form that can be obtained in this way is given in an appendix (Appendix III), but practically such speculations have little value for the following reasons:

(1) There are no statistics by which to test any hypothetical formula.

(2) There is no proof that the extent of an epidemic depends upon the number of primary foci of imported plague.

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It is indeed possible to seek an explanation upon different lines. For instance, it has been suggested that plague introduced among rats does not spread beyond the limits of the rat colony which received the infection from without. But, if village A with N inhabitants contains two distinct colonies of rats and village B with $\frac{N}{2}$ inhabitants only one colony, A is more likely to receive infection in one or other of its colonies than is B to suffer importation into its single colony. On the other hand, if B do get infected, the disease may be more widely disseminated through it than would be the case with a one-colony-infection in the larger village A. The principle here involved is precisely that which influenced relative mortality in favour of the small villages when we supposed the distribution of human plague to follow the simple binomial law (p. 79). I have given an arithmetical example above in dealing with that case; it will be interesting to consider the theory of the matter at a little more length in connection with the hypothesis just mentioned. It will, I believe, be found that any such interpretation is of doubtful validity.

Let p be the chance that plague will arise in a colony of rats and q the chance that it will not arise ($p+q=1$). Let us suppose that in a village with N human inhabitants there will be a single colony of rats and in a village with mN inhabitants m such colonies. Let us further assume that infection cannot be conveyed from one rat colony to another and that the number of human plague-deaths varies directly with the number of plague-stricken rat colonies so that if k human deaths are associated with one infected colony, mk deaths occur when m colonies are infected. If T villages each containing mN inhabitants and m rat colonies be exposed to risk, the average rate of mortality in the infected villages will be $\frac{pk}{N(1-q^m)} = \frac{(1-q)\,k}{(1-q^m)\,N}$[1].

The average rate in villages of N inhabitants is, of course, $\frac{k}{N}$ and the ratio of the rates accordingly $\frac{1-q}{1-q^m}$ which diminishes as m increases and for a constant value of m diminishes as q increases[2].

[1] The total number of deaths

$$= Tk\,(mp^m + (m-1)\,mp^{m-1}q + \frac{(m-2)\,m\,(m-1)}{2\,!}\,p^{m-2}\,q^2 + \text{etc.}) = Tmpk\,(p+q)^{m-1} = Tmpk.$$

The population of the infected villages $= TmN\,(1-q^m)$.

$\therefore$ rate of mortality in infected villages $= \frac{pk}{N\,(1-q^m)}$.

[2] Since it can be written $\frac{1}{1+q+q^2+q^3+\ldots q^{m-1}}$.

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Now we have seen an illustration of this principle in the case of the ordinary binomial distribution obtained by assuming that human deaths from plague could be treated as simple independent occurrences. It was pointed out in this connection that the value of p deduced from the actual statistics was too large for the observed differences in mortality rates to be explicable along these lines. But the p of our present hypothesis will be larger than that of our former supposition since otherwise we should reach the absurd conclusion that the number of human plague deaths corresponding to an infected colony of rats is not less than the number of persons corresponding to a rat colony in general[1].

It is further to be remarked that the fall in rate of mortality as the populations of villages exposed to risk increase is exaggerated by the hypothesis that either plague in different colonies of rats or plague in different human beings are really independent. We have supposed that when a single colony is infected there will be k human deaths and when m colonies are infected mk deaths. But it is far more likely that of two villages each containing the same population and the same number of rat colonies, say, m rat colonies, and the former having m infected colonies and the latter only one, that the mortality in the first will be more than m times that of the second. Using the notation already employed we have for the total mortality on the hypothesis of independence:

$$Tk\,(mp^m + m\,(m-1)\,p^{m-1}q + \ldots) \quad \ldots\ldots\ldots\ldots\ldots(1).$$

If we imagine some cumulative effect this will be changed into an expression of, *e.g.*, the form

$$Tk\alpha\,(\alpha^{-\frac{1}{m}}\,mp^m + \alpha^{-\frac{1}{m-1}}\,m\,(m-1)\,p^{m-1}q + \ldots) \quad \ldots\ldots\ldots(2),$$

where α is greater than unity.

(2) will always be greater than (1) unless $m = 1$. Consequently the rate of mortality in infected villages of populations mN each will be greater than before.

I am accordingly unable to find a satisfactory explanation of the

[1] Let N = total population of a district, Nk_1 the number of human deaths from plague, Nk_2 the number of colonies of rats in the district, h the number of human deaths per infected colony of rats, p_1 the independent chance of a colony being infected and p_2 the chance of a human plague-death. $p_1 = \frac{Nk_1}{hNk_2} = \frac{k_1}{hk_2}$ and $p_2 = k_1$. If $\frac{k_1}{hk_2} < k_1$, $\frac{1}{k_2 h_1} < 1$. $\therefore\ k_2 h > 1$. $\therefore\ h > \frac{1}{k_2}$. $\therefore$ no. of deaths per infected colony is greater than the average no. of persons per colony.

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facts in this way. It has seemed worth discussing the more plausible hypotheses at some length because they afford an excellent illustration of the difficulty which has to be faced in attempting to give an account of a striking phenomenon[1]. It would appear that we require the following additional statistics before an adequate theory can be propounded. We must know:

(1) Relation between the extent of an epidemic and the number of initial centres of plague.

(2) Relation between the human and rat population of villages of different sizes.

(3) Relation between the absolute population (human) and the density of population in villages.

We also require to know whether there is any relation between absolute size and prosperity, type of house, occupations of the villagers, etc. I am aware that such information is certainly difficult and perhaps impossible to obtain for an adequate number of villages. Without it, however, all I feel justified in concluding is that a difference can generally be observed between the rates of mortality in large and small villages from the region investigated and that this difference is no mere *simulacrum* or arithmetical will-o'-the-wisp, due to the employment of ratios. I conclude the section by expressing these results in summary form:

(1) *In nearly all the districts and epidemics studied the rate of mortality in small villages which became infected was higher than in large ones.*

(2) *This phenomenon cannot be regarded as a spurious effect due to the use of relative numbers or as an error of random sampling.*

(3) *If however the populations of villages not infected are included, there is no constant difference between the rates of mortality in large and small communities.*

V. *The influence of intra-seasonal variations in rainfall and temperature upon the rate of mortality in the villages under consideration.*

It is well known that the epidemic prevalence of plague is markedly seasonal, and the Commission have already studied the particular bearing of the general facts upon the epidemiology of plague in India[2]. With the general conclusions arrived at in the report cited in the

[1] See further in Appendix IV.

[2] *Journ. Hygiene*, VIII. 1908, p. 288 *et seq.*; also *ibid.* X. 1910, p. 446, etc.; see also Sticker, *Abhandlungen aus der Seuchengeschichte*, etc., Bd. I. Part 2, pp. 238, etc.

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footnote, the present data completely agree so that it is unnecessary to consider here that aspect of the subject. One topic, however, in connection with this matter has seemed worthy of notice on the present occasion. The constancy of the general type of seasonal prevalence in any one district shows that plague will only become epidemic when the meteorological conditions (or something highly correlated with these) fall within certain limits[1]. It may be asked how far variations *within these limits* will alter the course and extent of the epidemic. Thus, we generally find that the mortality registered in any two epidemics is not precisely the same, often the difference is very considerable; can this be attributed to the differences in weather conditions which are found from year to year? Again, suppose that April is the month which contributes the greatest proportion of plague deaths, shall we find that the variations in the April quota from epidemic to epidemic can be brought into relation with changes in the average weather conditions of that month?

Complete data for answering these questions do not exist. In the first place we have the monthly plague figures separately recorded for only a few towns and villages; in the second, although we have numerous rainfall statistics and records of temperatures at a few places, no observations on percentage humidity are available for the districts under examination. Such data as are to be found have been summarised in Table XXXIX. Special details are given for Amritsar city in Table XL. Considering the relation of the course of several epidemics in Amritsar city and the temperature curve for Lahore, the conclusion to be drawn from a study of the facts is, I think, that both our questions must be answered in the negative. It does not seem that the relatively small changes from year to year in meteorological conditions within each part of the epidemic season can be regarded as of much importance as mortality factors. This general impression is confirmed by some applications of the method of correlation, all of which yielded negligible results. We must not hastily conclude from this that such variations are in fact without importance, but merely that their weight, in comparison with other factors, is not sufficient to reveal itself in the present kind of material. Study of the work published already by the Commission will convince the reader that the meteorological factor is of a complex nature. Its complete elucidation will require applications of the method of multiple correlation, for which the immediate statistics are not suitable.

[1] Some statistical points relative to the seasonal prevalence are examined in Appendix II *infra*.

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VI. *On local differences in mortality.*

It will already have been noticed, in earlier tables, that the rate of plague mortality in villages infected in the same year, approximately equal in size and returning cases in the same cycle of months, exhibits not inconsiderable variations from district to district; within the same district, indeed, marked differences can be seen. This very important question will now be studied. Tables XLI–LVIII exhibit the rates of mortality in the different tehsils, or administrative subdivisions, of each district. Some notes on these tables are now given.

Hoshiapur. 1901–2. Una tehsil had a lower mortality than the others, but the whole outbreak was a small one.

1903–4. The same remarks apply to this year.

1904–5. Although a considerable number of villages in Una were at risk, the rate of mortality is still low.

1906–7. Una exhibits a lower rate of mortality than Garshankar or Dasuyah, but the same rate as Hoshiapur from which, paying attention to average size of village and length of exposure, it cannot be distinguished. The general experience of this district is that Una, which is separated from the rest of the district by a continuous range of hilly country, suffered less than the other tehsils. The difference is not however very striking.

Amritsar. 1902–3. Tarn-Tarn appears to have suffered more than either of the other tehsils, notably more than Ajnala notwithstanding the latter's lower average population.

1903–4. In this epidemic Ajnala is also relatively immune.

1904–5. Tarn-Tarn again takes the lead, the general picture being similar to that of 1902–3.

1905–6. Amritsar tehsil had many more infected villages than the other tehsils, also the heaviest rate of mortality.

1906–7. The features of 1903 and 1905 are reversed, the average population of Ajnala villages is however much less than that of the others. Evidently no general conclusion can be drawn as to the experience of this district which varied from year to year.

Rohtak. 1904–5. In view of its low average population, Jhajjar tehsil escaped lightly.

1906–7. The same remark applies again but very few Jhajjar villages were at risk.

Both the epidemics in Rohtak exhibit marked local divergences, a circumstance which must be taken into account when we remember

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the apparent exception presented by this district to the general rule of higher mortality rates in smaller villages (*vide supra*). I do not however suggest that this is a complete explanation.

Mozaffarnagar. No appreciable local differences are to be found in this district.

Gujrat. 1903–4. Phalia tehsil exhibits an *enormously* greater mortality than the other tehsils, but also had a much larger number of villages at risk.

1904–5. No really significant differences in the local rates.

1906–7. This epidemic is the most interesting in the series because numerous villages were infected all over the district. The rate in Phalia is once again very much heavier than in Gujrat and, having regard to average population, appreciably heavier than in Kharian. Gujrat district suggested such marked local peculiarities in the incidence of plague that it was investigated in further detail. We notice in 1903–4, a decided maximum in the south-west part of the district. In 1904–5 the maximum has passed over in a north-east direction to the Gujrat tehsil. In 1905–6 less than 70 villages in all were infected, 32 in Gujrat, 12 in Kharian and 25 in Phalia, the respective rates being 1·3, 1·3 and 2·7 %. In 1906–7, the wave sweeps round to the north-west, Kharian being deeply submerged. As I have said, this year is particularly important because the average populations and total numbers of villages infected are not very different in the three tehsils. To get more exact notions, a map was prepared. Circles were marked on a map of the district to show villages which had returned deaths corresponding to a rate of three times (or more) the average mortality for the epidemic. It will be noticed that a line roughly parallel to the main railway and seven or eight miles south-west of it would leave considerably more than half the "heavily infected" villages and rather less than half the total area below and to the south-west. The most striking concentration, however, relates to the big epidemic of 1906–7 alone. The large circle on the map has its centre at Dinga railway station and a radius of ten miles. Within its circumference were 122 infected villages the populations of which could be ascertained; in the rest of the district 729 villages were infected. The average mortality within this selected area reached the enormous figure of 17·3 %, elsewhere it was less than half this. Further, only 6·6 % of the villages reported deaths as early as February while 11·5 % of the remainder were infected in this period. Some details appear in Tables LIX–LX. I think these differences are too considerable to be fairly attributed

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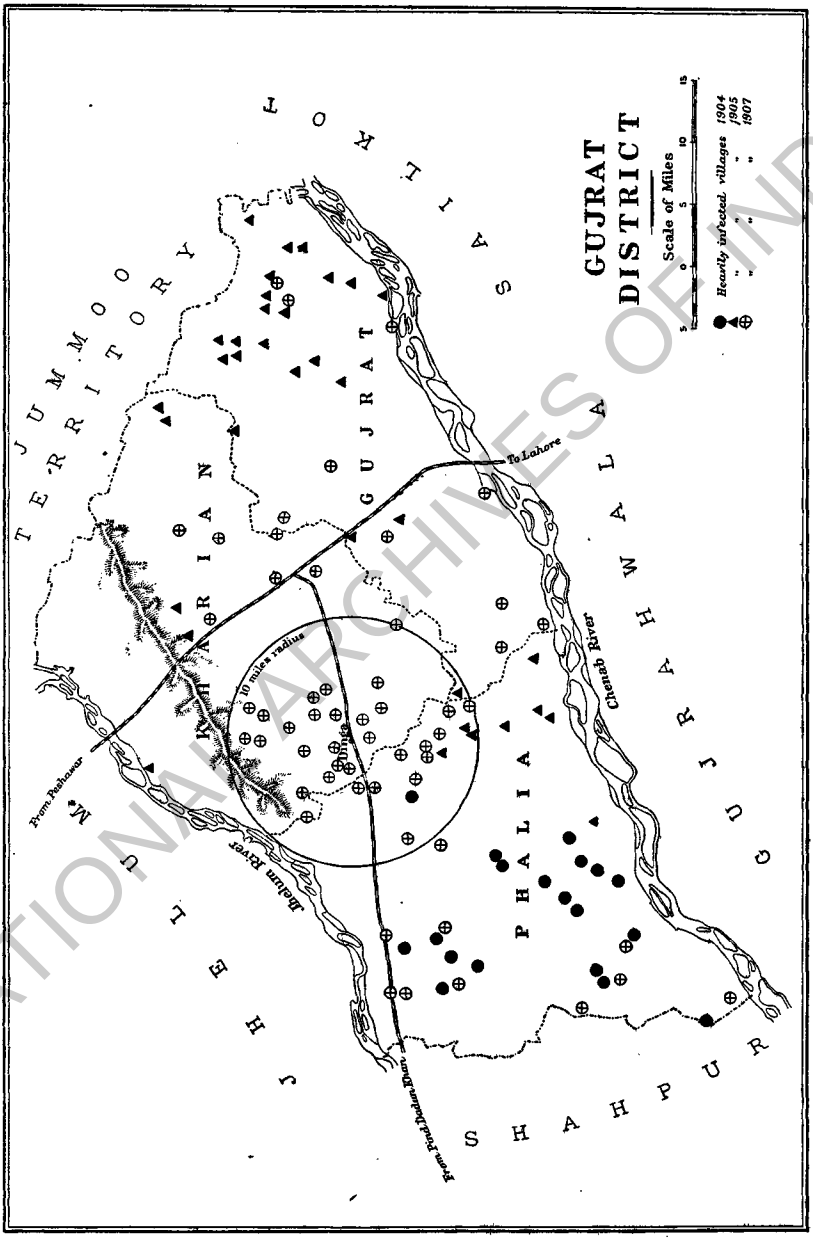

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either to errors of record or of random sampling. It would seem that some local influence was here at work which specially favoured plague; of the nature of this factor, nothing can be gleaned from the statistics, so that it is not worth speculating here about the point, which, however, deserves attention. I think it must be admitted that some, unascertained, local peculiarities are of considerable significance, but that these factors are not necessarily constant from epidemic to epidemic. This will be seen from a general comparison of one district with another.

If we compare the districts for the three epidemics of a wide-spread character we have the following results:

District	Percentage mortalities in infected villages		
	1903—4	1904—5	1906—7
Gujrat	10·1	4·6	9·5
Rohtak	—	6·0	7·0
Amritsar	3·7	4·4	3·8
Mozaffarnagar	2·4	3·2	6·7
Hoshiapur	4·6	3·0	2·6

The differences as between the districts for the same epidemic are considerable. It will be noticed also that Gujrat and Rohtak, the districts within which we have seen the greatest local divergences in two out of the three epidemics were severely affected. Gujrat never takes a lower place than second in the order of severity. The general conclusions which seem to follow from the data here analysed can conveniently be grouped under six headings.

(1) The virulence of an epidemic in any one district varies greatly from year to year.

(2) This is partly due to some general phenomenon since the tendency is for the mortality to be high in all districts for a given epidemic or conversely.

(3) The influence is however partly local since the relative positions of the districts in respect of mortality rate are not quite the same from year to year.

(4) The importance of local factors is also demonstrated by the irregularity of incidence within any one district. An irregularity too great to be attributable to simple differences in average size, or length of exposure.

(5) The local factors, the sum of which makes in favour of a high rate of mortality, cannot be permanent in character since the subdivision

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which has the heaviest mortality in one epidemic does not invariably occupy the same position in a subsequent outbreak.

(6) The rate of mortality is not necessarily heaviest in that subdivision which, judging from the number of infected villages, has plague most widely disseminated through it.

VII. *Summary of statistical conclusions.*

The detailed discussion of the conclusions here collected will be found in previous sections, together with the cautions necessarily to be borne in mind when weighing them (pp. 64, 65).

(1) Large villages tend to be infected earlier in the plague season than small ones.

(2) In villages of the same size total mortality is appreciably correlated with length of exposure to infection. The intensity of the relation, although quite appreciable, is not sufficient to account for the large differences in mortality rates noticed in villages of about the same size. There is some reason to think that villages infected before the months in which plague, owing to seasonal conditions, is most prevalent, do not suffer very appreciably more than villages not exposed until March or April.

(3) In nearly all cases, large *infected* villages suffer less than small ones.

(4) Plague does not occur at all in epidemic form during July, August and September in the districts analysed. The annual variations in temperature and rainfall observed during the epidemic season are not appreciably associated with variations in the rate of mortality.

(5) Differences in plague mortality in different districts, which are extremely marked, do not seem to be associated with climatic distinctions.

(6) Within any one district the variations in the rate of mortality observed in different subdivisions are considerable.

(7) A high rate of mortality in any subdivision is not necessarily accompanied by a wide dissemination of plague.

(8) There is no evidence that certain districts are *permanently* subject to a high rate of mortality. Local conditions making for an unusual degree of severity appear to vary from epidemic to epidemic.

(9) The rate of plague mortality in a village is seen to depend upon three factors, (*a*) length of exposure to infection, (*b*) number of inhabitants, (*c*) situation. Of these (*c*) is probably the most important; how it acts, however, cannot be determined from the present material.

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VIII. *Some general remarks on the epidemiology of plague with special reference to village communities.*

In the following paragraphs, I shall epitomise some general considerations partly examined in the present and former reports. By adopting this course I hope to place the essential elements of the problem in a clearer light than was possible during the examination of a mass of statistical details.

The investigation of plague from the epidemiological side resolves itself into an attempt to answer three questions:

(1) How does the disease enter a given country or district?

(2) Having effected an entrance how does it maintain itself there?

(3) What circumstances determine the transformation from endemic to epidemic prevalence and conversely?

(1) The majority of epidemiologists hold, I believe, that the origin of the present pandemic, the 1896 outbreak in Bombay, was probably plague ship-borne from Hong-Kong, but the point cannot be regarded as settled[1].

Any identification results from the weighing of probabilities somewhat delicately balanced and complete unanimity need not be expected. If we accept the hypothesis that plague always arises from pre-existent plague, a probable but not demonstrable supposition, it is to be remarked that its implantation evidently requires the co-operation of various factors the nature of which is still obscure. It is, perhaps, generally thought that the great pandemic revivals, *e.g.* that of the sixth century, the Black Death and the present outbreak depend in the last resort upon a special accession of virulence to the specific organism. This cannot, however, be a complete explanation since, for instance, the Provence plague of 1720–1, judging from its rate of mortality, was more virulent than the worst in our Indian experience; but it did not possess wide dispersive power. In other words, virulence and diffusibility are not interchangeable expressions. This part of the epidemiological subject does not, however, specially concern the phenomena of village plagues.

(2) That the intra-local spread of human plague is due to an epizootic cannot be seriously questioned so far as India is concerned. Nevertheless we are not warranted in asserting that this method of

[1] See *Report of Indian Plague Commission*, 1901, v. p. 7; *The Present Pandemic of Plague*, by J. M. Eager, Washington, 1908; E. H. Hankin, *op. cit.*; G. Sticker, *op. cit.* Part 1, p. 354; W. J. Simpson, *Treatise on Plague*, 1905, pp. 66, etc.

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spread has prevailed in all epidemics elsewhere, even when the type was predominatingly bubonic. For instance, in many parts of Europe, probably in English rural districts, the Black Death was chiefly bubonic plague, but I can find no trustworthy evidence that a rat epizootic was a factor in this particular pandemic.

In the Punjab, plague is maintained as a smouldering fire in some villages during the off-season. These villages generally exceed the average size, in some cases considerably. Apart from the fact that the likelihood of infection increases with size, other considerations tend to explain this, such for instance as the greater chance of persistent infection in an absolutely large than in a small population of rats.

It must of course be clearly understood that the statistical results obtained by Major Lamb and myself do not warrant us in asserting that *all* village plagues are started by importation from a large centre; but we are justified in affirming that this method of origin has been in all probability the most usual process. Hence, our answer to (2) is briefly—plague maintains itself in towns, not to any great extent in villages. Whether centres of occupation on an endemic level in the towns themselves require re-inforcement from a permanent focus of plague, is an epidemiological problem not needing consideration at this place.

(3) Our answer to (3) in broad outline rests upon remarkably full and concordant information. We see in the Punjab, in harmony with the experience of other countries and ages, that the first pre-requisite of an epidemic is the realisation of certain conditions of temperature and humidity. In the case of villages having no plague in the off-season, the probability of an epidemic happening at all depends upon (α) size; *other things being equal*, the likelihood varies inversely as size. This inter-dependence is not, of course, direct, *i.e.* a village of population n is not just twice as likely to have an epidemic as one of population $\frac{1}{2}n$, but increase in the percentage of infected villages as the average population rises is substantial: (β) proximity to lines of transit; this being, in some but not all districts, evident when one studies the prevalence in villages near a railway. The *severity* of the epidemic in infected villages will be maximal when (a) importation occurs early in the plague season, (b) the village is a small one. I hope to have made it seem probable that this condition depends upon some material circumstance differentiating communities of different sizes. Since, so far as I am aware, no evidence of a comparable character has been collected with the object of determining whether the same rule

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holds in the case of other epidemic diseases, such as cholera or enteric fever, it cannot be stated that (*b*) applies to plague alone. The historical evidence *suggests* that the rule may be of general application to epidemics of plague but cannot be regarded as of probative force. (*c*) We must also include, as a factor regulating severity, some local condition, constant for one epidemic but variable from epidemic to epidemic, which tends to raise or lower the mortality rate in contiguous hamlets irrespective of the numbers of their inhabitants.

It would naturally be expected that a severe epidemic would tend to exhaust the susceptible persons and rats so that severe and mild outbreaks would alternate. It might also be anticipated that dispersive and toxic powers would be, *to some extent*, associated so that epidemics affecting a large number of villages might have the highest rates of mortality. These surmises are only in part justified by the statistics collected in earlier chapters of the report (*e.g.* Table X). There is no marked regularity in the succession of severe and mild epidemics and the outbreak affecting the largest number of villages has not always—although usually—shown the highest mortality rate. It would not be safe to predict that a province seriously ravaged in one year will escape lightly in the following season.

With regard to the natures of factors comprised under (*c*), little can be said. It seems, however, improbable that climatic changes are responsible. I hope to have proved that variations both inter- and intra-local in the mortality rate are too large to be accounted for in this way. The same remark applies to changes from year to year; the exhaustion of susceptible persons and rats plays some, possibly a considerable part; climatic changes play a less, probably much less, important *rôle*; there remains a *tertium quid* which is not apt to be placed in evidence by statistical inquiries based on existing data.

I may be permitted to hope that the analyses communicated in this report have thrown light upon some points in the epidemiology of plague in a district containing scattered villages. I recognise that, in certain directions, it has been impossible to attain results of a character sufficiently definite to warrant one in attaching much importance to them. Even in these instances, however, it is possible that the labour has not been in vain because a first step towards the attainment of definite knowledge is a summary of the imperfections in data already collected.

In conclusion I would remark that the lamented death of Major George Lamb has deprived this report of much of the value it might

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otherwise have possessed. The loss of his advice and criticism in the reduction of data for the most part prepared by him has been a serious blow, and I have particularly missed that wide knowledge of local circumstances which he had at his command.

Appendix I.

A note on town and village mortality rates from English plague records.

With the exception of an outbreak in "Cadwallader's Time" and a doubtful record for 1010–11, English plague experience is confined to a period of some 350 years, commencing with the importation of the Black Death, probably at Weymouth in 1348, and ending a few years after the Great Plague of London in 1665. I have brought together in this note such scanty evidence as I have found bearing upon the relative incidence of the disease upon different population groups.

(1) The Black Death. The rate of progress of the disease can be measured in London by a comparison of wills enrolled in the Hustings Court during 1348–9 with the average for other years. Such a comparison suggests that the mortality curve reached its maximum in April–May 1349. This does not correspond to the subsequent seasonal experience of London and may perhaps be explained by a tendency towards a pneumonic type. With regard to the actual figures, Robert of Avesbury asserts that upwards of 200 burials took place daily in Manny's cemetery before Easter, not to speak of interments elsewhere. Creighton infers that the maximum daily average may have reached 200, *i.e.* about that of the worst weeks in 1563. This would give a total mortality between 20,000 and 30,000, a rate of some fifty per cent. On the basis of Seebohm's data, it would appear that village mortality surpassed this figure (*vide infra*). Some indications point to the rate having been higher in Norwich than in London. Thus, before the Black Death, the proportion of men-at-arms levied on Norwich and London were in the ratio of 6 to 10 (writ of 1351 seemingly based on a pre-1349 standard). This is a rough measure of the ratio of the populations and confirmed by the numbers of ecclesiastical parishes in the two towns (120 in London, 60 in Norwich). Thirty years later, after the Black Death and several severe outbreaks subsequently, the populations were in the ratio of 17 to 100 (estimated from the Poll Tax returns of 1377). It may be that the relative decay of Norwich is

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partly attributable to the ravages of plague which seems often to have been especially severe in the Eastern counties.

Turning now to the general rate of mortality all over England (see Table LXI), two methods of inquiry have been pursued, viz. an analysis of the number of institutions in different dioceses, and a study of Manor Court Rolls. Some of the figures relating to institutions of parochial clergy, due to the researches of Jessopp and Gasquet, appear in Table A. It would seem that in some districts the mortality was greater than sixty per cent. and there is no sign of a lower rate in the sparsely populated northern districts. With respect to village mortality, Jessopp's well-known results should be mentioned. In the manor of Cornard Parva, near the boundary of Essex and Suffolk, the tenants are estimated to have been less than fifty; in six months, 21 families were obliterated; sixty deaths are noticed in the Manor Rolls, this number not including all minors and dependents.

Similar evidence is available in the cases of Hunstanton and Croxford. Jessopp concludes that "during the year ending March 1350, more than half the population of East Anglia was swept away by the Black Death. If any one should suggest that *many more* than half died, I should not be disposed to quarrel with him" (Jessopp, p. 206). Seebohm, who investigated the rolls of the manor of Winslow, states that 153 holdings changed hands during the plague; of 43 jurors who had served in 1346–8, 27 died in 1349, a mortality of over 60 per cent. (Seebohm, p. 29). If these results are typical, it seems clear that the village rate was not less and may have been more than that of London[1].

I think the general impression to be obtained from a study of the fourteenth century pandemic is that, from the point of view of relative mortality, there is some similarity between its course and that of Indian experience; the similarity does not, however, extend to other epidemiological factors.

(2) When we come to the sixteenth and later centuries, at least one important epidemiological distinction is to be noticed. While some evidence, for example the Paston letter cited by Creighton (Creighton, p. 226), suggests that plague may have been endemic in country districts during the fifteenth century, there is no evidence of this being the

[1] The contemporary Simon de Corvino, in a poem on the Black Death, writes: "Et nimis immensum sensere suburbia damnum" (Haeser, III. p. 172). This may point to the mortality in small towns having been excessive, but the word *nimis* may have been introduced as a rhetorical expletive. I do not think we can, on the strength of this passage, cite de Corvino as an authority for the view that the Black Death was really more fatal in villages and small towns.

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case in more recent times. The available facts suggest that village plagues in the sixteenth and seventeenth centuries arose by importation from towns. The question is whether the rates of mortality also contrasted. In Table LXII I have collected some of the less unreliable figures and the following records may be compared with them. In the years 1578–9, we have for London 4197 deaths (Jan. 1578–Dec. 1579), from April to December 1578, 3354 deaths. At Norwich from Aug. 20th 1578–Feb. 19th 1579, 4817 are recorded, 2335 English and 2482 aliens, giving a much heavier rate. Two thousand deaths are also reported in Yarmouth. In London during the plague year 1593, 17,844 was the total number of deaths, 10,662 from plague; in 1595 there were 3507 deaths from all causes so that the ratio of deaths in an ordinary year to deaths in a plague year would be 1 : 5·1. The burials in one parish of Shrewsbury during the plague year are in the ratio of 7·3 : 1, unity being the burials in an ordinary year. This suggests a rate certainly not below that of London. At the same period, more than 1100 deaths from plague are said to have occurred in the small city of Lichfield, but round figures of this sort are notoriously unreliable. In the town of Kirkoswald, plague appeared in 1598; the burials in that year were 583, in 1597 they numbered 42.

The famous epidemics of 1603, 1625, and 1665 tell the same story (see Table B). In the provinces, between the London plagues of 1625 and 1636, some towns experienced epidemics which would rank with the great London plagues in point of mortality rate, *e.g.* Newcastle (Creighton, p. 529).

(3) In Table LXIII I have collected data bearing upon the mortality rates of the epidemic in the South of France, 1720 (Sticker, 229). These results are indefinite but not irreconciliable with English experience.

Upon the whole, making due allowance for the fragmentary character of the material, it would appear probable that the rule deduced from the Punjab village statistics, viz. that the rate of plague mortality tends to increase as the absolute population of the infected community diminishes, may apply to European epidemics. If this be accepted, then, since it is doubtful whether the spread of the disease in the Black Death pandemic can be safely attributed to an epizootic, and it is probable that the material conditions of life in villages and small towns were inferior to those of the larger and more prosperous corporate cities, some support is afforded to the suggestion that material rather than numerical or purely epidemiological factors are responsible for the Punjab figures. It is unlikely that methods of sanitary administration, in the strict sense, can have had much to do with the results.

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APPENDIX II.

Note on the epidemic curves of Punjab districts.

While the general form of the epidemic curve is well known and has been frequently illustrated in the Advisory Committee's reports, it appeared worth while answering two questions.

(1) Does the form vary from one district to another?

(2) Is there any difference in the form of a severe as compared with that of a mild epidemic?

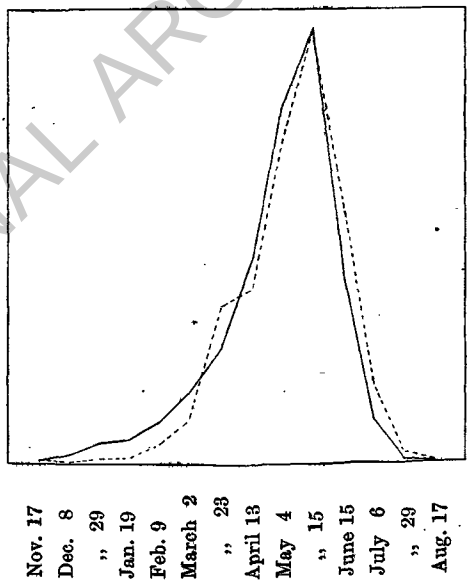

Fig. 3.

——— Amritsar. ----- Gujrat.

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I chose the 1906–7 epidemics for Amritsar and Gujrat districts as examples of severe outbreaks in a mainly agricultural (Gujrat) and partly urban (Amritsar) territory; 1905–6 Amritsar district illustrates the course of a relatively mild epidemic. The figures were obtained from the weekly official returns made to the Government of India. In

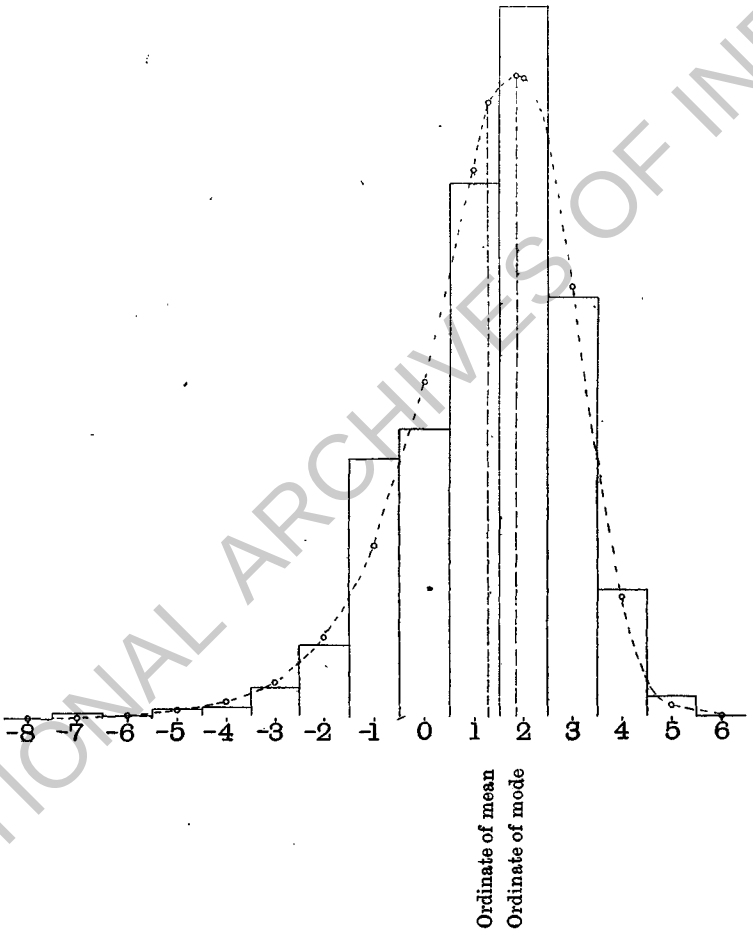

Fig. 4. Base unit, 3 weeks (the numerals refer to an arbitrary origin). Ordinate = no. of deaths from plague in each period of 3 weeks from Sept. 1906 to Sept. 1907, Gujrat. The rectangles represent the actual observations. The equation of the dotted curve is:

$$y = 1{\cdot}1724\left(1+\frac{x^2}{(3{\cdot}8854)^2}\right)^{-8{\cdot}6661} \times e^{-19{\cdot}5399\tan^{-1}\frac{x}{3{\cdot}8854}}.$$

Origin at 6·2395.

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the case of Amritsar, I have not entered the figures for Amritsar city when separately given.

As will be seen from the figures (Table LXIV) and diagram (Fig. 3) (in the diagram the two sets of figures are reduced to the same total) the course of the Gujrat epidemic almost exactly corresponds with that of Amritsar district. The asymmetry is marked, the fall being steeper than the rise. The minor epidemic of 1905–6 does not appear to differ in form from the wide-spread attacks.

The three sets of figures were analysed by Pearson's method. The Gujrat frequency proved to be of Type IV, Amritsar 1906–7 of Type I and Amritsar 1905–6 of Type VI. In each case the fit was poor although the Gujrat curve, which is reproduced (Fig. 4), is a tolerable representation of the course of events from the graphic standpoint. I think it is very doubtful whether the frequencies are homogeneous; there is certainly no ground for regarding them as even approximately symmetrical. In the result, so far as the data go, both questions proposed must be answered in the negative. The practical conclusion is that the factors which cut an epidemic short are more determinate than those responsible for its commencement—an inference warranted by all our other evidence.

Appendix III.

Note on possible interpretations of the curve of relative mortality.

It is not difficult to frame hypotheses which would account for changes in relative mortality with size similar to those discussed in the text. The following is given as an elementary illustration; it is to be clearly understood that the assumptions made do not rest upon any reliable basis.

If the two factors which influence the rate of mortality be (1) number of initial plague centres, (2) density of population.

If, further, (1) increases more slowly than the absolute population, and if (2) increases with the population to a constant value, then

$$y = A\alpha^{\frac{k}{n+1}}\beta^{-\frac{1}{n}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

is a possible form, where y = the rate of mortality, n the number of inhabitants per village and α, β, k and A are constants.

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If, for simplicity, we put $\alpha = \beta$ we have

$$y = A\alpha^{\frac{n(k-1)-1}{(n+1)n}} \quad \text{.....(2).}$$

Differentiating (2) we find that y has a maximum when $n = \frac{-1 \pm \sqrt{k}}{1-k}$.

The diagram (Fig. 5) which gives part of the curve $y = 10^{\frac{3n-1}{(n+1)n}}$ indicates a state of affairs suggestive of the actual facts. The nature of the assumptions does not warrant us in attaching much significance to the results. The subject is, however, of interest and might be worth further investigation along these lines.

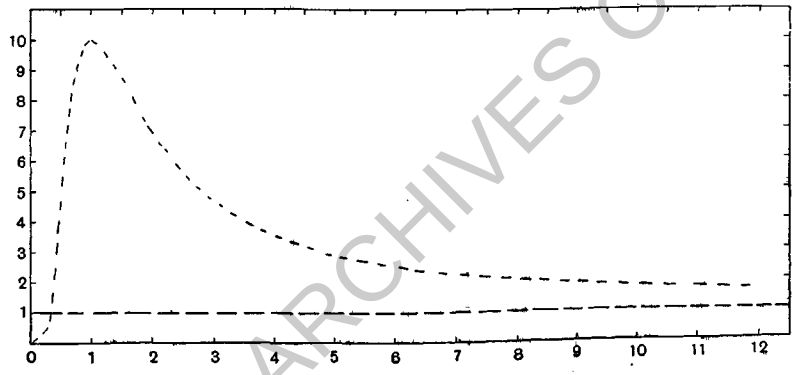

Fig. 5.

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TABLE I.

ROHTAK 1905.

Populations 200—250.

Population	Actual number of deaths	Calculated number
200	20	20·29
209	75	21·21
211	5	21·41
216	6	21·92
224	21	22·73
231	4	23·44
1291	131	131·00

Populations 600—650.

603	10	22·77
606	23	22·89
609	38	23·00
612	41	23·12
622	6	23·49
634	39	23·95
637	20	24·06
645	31	24·36
645	4	24·36
5613	212	212·00

Populations 950—1000.

951	27	77·71
954	105	77·96
956	36	78·12
957	98	78·21
964	72	78·78
974	72	79·59
981	73	80·16
997	149	81·47
7734	632	632·00

ROHTAK 1905.

Populations 650—700.

Population.	Actual number of deaths	Calculated number
656	1	59·79
664	130	60·52
663	60	60·43
669	9	60·98
675	67	61·52
677	173	61·71
680	11	61·98
681	38	62·07
5365	489	489·00

Populations 700—750.

729	33	45·31
725	20	45·06
723	49	44·94
721	52	44·81
710	61	44·13
706	29	43·88
705	70	43·82
741	44	46·05
5760	358	358·00

Populations 750—800.

751	103	39·73
759	18	40·15
766	31	40·52
782	48	41·37
796	29	42·11
796	17	42·11
4650	246	245·99

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TABLE II.

ROHTAK 1905.

Populations 1400—1500.

Population	Actual number of deaths	Calculated number
1409	92	73·05
1415	161	73·36
1431	176	74·19
1432	90	74·25
1445	12	74·92
1467	28	76·06
1468	85	76·11
1474	21	76·43
1478	10	76·63
13,019	675	675·00

Populations 1650—1700.

Population	Actual number of deaths	Calculated number
1658	186	115·33
1660	99	115·47
1661	60	115·54
1671	178	116·23
1681	143	116·93
1686	16	117·28
1698	195	118·11
1698	56	118·11
13,413	933	933·00

TABLE III.

ROHTAK 1905.

Populations 350—400.

Population	Actual number of deaths	Calculated number
354	4	22·32
356	33	22·45
357	18	22·51
359	97	22·64
362	7	22·83
363	3	22·89
364	21	22·95
369	7	23·27
372	37	23·46
380	5	23·96
383	19	24·15
391	31	24·66
393	6	24·78
396	30	24·97
399	35	25·16
5598	353	353·00

Populations 250—300.

266	5	12·92
267	2	12·97
267	11	12·97
269	16	13·07
273	1	13·26
275	8	13·36
277	13	13·46
286	25	13·89
292	26	14·19
296	18	14·38
299	24	14·53
3067	149	149·00

ROHTAK 1905.

Populations 400—500.

Population	Actual number of deaths	Calculated number
400	207	39·34
410	33	40·32
413	15	40·61
418	38	41·11
419	3	41·20
423	33	41·60
435	55	42·78
437	30	42·97
443	23	43·57
450	6	44·25
453	21	44·55
457	91	44·94
485	31	47·70
488	40	47·99
499	26	49·07
6630	652	652·00

Populations 500—550.

511	50	18·52
513	20	18·59
517	13	18·74
525	8	19·03
526	4	19·06
526	18	19·06
3118	113	113·00

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TABLE IV.

ROHTAK 1905.

Populations 1150—1200.

Population	Actual number of deaths	Calculated number
1155	103	77·76
1160	45	78·09
1171	19	78·83
1173	87	78·97
1177	98	79·24
1178	165	79·30
1181	61	79·51
1188	57	79·98
1191	78	80·18
1192	63	80·25
1193	54	80·31
1197	123	80·58
14,156	953	953·00

Populations 1200—1300.

1298	1	77·45
1296	76	77·33
1297	6	77·39
1285	39	76·68
1285	7	76·68
1236	275	73·75
1228	119	73·28
1214	82	72·44
10,139	605	605·00

ROHTAK 1905.

Populations 1000—1100.

Population	Actual number of deaths	Calculated number
1004	93	52·80
1014	96	53·32
1031	43	54·22
1035	38	54·43
1042	11	54·79
1059	6	55·69
1059	79	55·69
1060	3	55·74
1064	128	55·95
1077	115	56·63
1084	96	57·00
1086	14	57·11
1092	10	57·42
1095	8	57·58
1096	96	57·63
15,898	836	836·00

Populations 1100—1150.

1102	65	66·62
1104	59	66·74
1112	102	67·23
1113	26	67·29
1141	49	68·98
1141	125	68·98
1144	49	69·16
7857	475	475·00

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TABLE V.

AMRITSAR 1904.

Populations 700—750.

Population	Actual number of deaths	Calculated number
750	6	15·49
750	16	15·49
715	21	14·77
719	9	14·85
714	22	14·75
715	1	14·77
730	34	15·08
711	8	14·69
734	1	15·16
746	56	15·41
749	18	15·47
721	11	14·89
716	19	14·79
727	2	15·02
707	17	14·60
715	4	14·77
707	29	14·60
743	8	15·35
729	3	15·05
13,798	285	285·00

AMRITSAR 1903.

Populations 700—750.

Population	Actual number of deaths	Calculated number
750	33	33·11
750	6	33·11
719	10	31·75
740	15	32·67
711	14	31·39
727	30	32·10
727	55	32·10
746	27	32·94
703	58	31·04
721	50	31·83
716	49	31·61
727	18	32·10
707	55	31·22
715	60	31·57
721	40	31·83
749	11	33·07
713	29	31·48
707	10	31·22
726	41	32·05
743	30	32·81
14,518	641	641·00

TABLE VI.

GUJRAT 1907.

Populations 450—500.

Population	Actual number of deaths	Calculated number
467	1	46·79
496	73	49·70
479	37	47·99
467	34	46·79
476	27	47·69
474	4	47·49
460	28	46·09
469	2	46·99
491	29	49·20
459	2	45·99
459	2	45·99
498	6	49·90
467	17	46·79
454	35	45·49
463	32	46·39
472	42	47·29
496	134	49·70
468	100	46·89
458	18	45·89
467	174	46·79

GUJRAT 1907 (*cont.*)

Population	Actual number of deaths	Calculated number
475	2	47·59
499	21	50·00
493	70	49·40
499	19	50·00
456	20	45·69
457	188	45·79
490	23	49·10
453	18	45·39
458	54	45·89
497	170	49·80
461	68	46·19
475	41	47·59
466	60	46·69
466	75	46·69
473	33	47·39
478	114	47·89
490	22	49·10
500	21	50·10
498	45	49·90
462	64	46·29
456	23	45·69
19,442	1948	1948·01

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TABLE VII.

GUJRAT 1907.

Populations 750—800.

Population	Actual number of deaths	Calculated number
794	11	90·81
771	7	88·18
767	98	87·73
768	103	87·84
758	5	86·70
775	145	88·64
787	62	90·01
785	135	89·79
779	220	89·10
755	295	86·35
781	62	89·33
764	27	87·38

GUJRAT 1907 (*cont.*)

Population	Actual number of deaths	Calculated number
769	77	87·95
767	270	87·73
760	70	86·93
753	66	86·12
770	43	88·07
797	31	91·16
778	67	88·98
786	89	89·90
794	16	90·81
752	60	86·01
796	66	91·04
775	94	88·64
785	96	89·79
19,366	2215	2214·99

TABLE VIII.

HOSHIARPUR 1902.

Populations 700—750.

Population	Actual number of deaths	Calculated number
722	21	20·46
707	27	20·03
745	42	21·11
717	31	20·32
742	4	21·02
729	4	20·65
748	36	21·19
703	7	19·92
746	30	21·14
739	21	20·94
749	5	21·22
8047	228	228·00

HOSHIARPUR 1907.

Populations 700—750.

Population	Actual number of deaths	Calculated number
739	2	21·97
712	15	21·17
748	1	22·24
729	14	21·68
746	19	22·18
709	20	21·08
747	9	22·21
739	13	21·97
742	62	22·06
711	14	21·14
725	116	21·56
711	1	21·14
722	2	21·47
707	6	21·02
711	14	21·14
706	34	20·99
745	36	22·15
734	11	21·83
13,083	389	389·00

NATIONAL ARCHIVES OF INDIA

TABLE IX.

General Statistical Account of the five Districts.

RELIGIONS.

District	Total population		Hindu	Percentage of total	Sikh	Percentage of total	Mohammedan	Percentage of total	Others	Percentage of total
Amritsar	1,023,828	100·0	280,985	27·4	264,329	25·8	474,976	46·4	3538	·4
Rohtak	630,672	100·0	533,723	84·6	94	—	91,687	14·6	5168	·8
Gujrat	750,548	100·0	69,346	9·2	24,893	3·3	655,838	87·4	471	·1
Hoshiarpur	989,782	100·0	603,710	61·0	71,126	7·2	312,958	31·6	1988	·2
Mozaffarnagar	877,188	100·0	606,833	69·2	280	—	255,292	29·1	14,783	1·7

OCCUPATIONS.

District	Pasture and Agricultural	Percentage of total	Unskilled Labour (not Agricultural)	Percentage of total	Provision of Food, Drink, &c.	Percentage of total	Provision of Textile Fabrics, Furs, &c.	Percentage of total	Personal and Household Services, &c.	Percentage of total	Others	Percentage of total
Amritsar	409,690	40·0	19,005	1·9	62,242	6·1	101,467	9·9	165,611	16·2	265,813	25·9
Rohtak	385,194	61·1	7626	1·2	20,567	3·3	35,242	5·6	40,127	6·3	141,916	22·5
Gujrat	476,177	63·4	14,913	2·0	27,351	3·6	47,614	6·3	45,130	6·1	139,363	18·6
Hoshiarpur	594,180	60·0	20,898	2·1	29,296	3·0	84,354	8·5	60,974	6·2	200,080	20·2
Mozaffarnagar	449,260	51·2	98,804	11·3	50,143	5·7	53,109	6·1	86,200	9·8	139,672	15·9

TRADE.

There are no District Statistics of imports and exports and the general information as to the trade of separate districts is meagre.

Amritsar exports wheat, shawls, carpets, cotton goods, jewellery and brass vessels, and imports (partly for re-export) grain, pulses, sugar, oil, salt, tobacco, raw cotton, cotton goods and yarn, wool, silk, shawls, blankets, metals, hardware, glass and dyes.

Rohtak exports cereals.

Gujrat exports wheat, millet, oil-seeds, oil, *ghi*, wool, cotton, cotton goods and hides, and imports piece goods, iron, sugar, salt, rice, wool, brass vessels, spices and dyes.

Hoshiarpur exports rice, grain, wheat, barley, sugar, hemp and other fibres, tobacco, indigo, cotton and lac, and imports cotton and cotton yarn, piece goods, millet, cattle, dyes, salt, sugar, tobacco, kerosine, and oil-seeds.

Mozaffarnagar exports wheat, raw sugar, barley, millet, rice and oil-seeds.

NATIONAL ARCHIVES OF INDIA

TABLE X.

Particulars of all Epidemics in each District.

District	Year	Total population	Number of villages infected	Total plague mortality	Death rate per cent.	Average population of infected villages
Hoshiarpur	1898	17,438	16	439	2·52	1089·9
,,	1899	3701	4	9	·24	925·3
,,	1900	9636	11	50	·52	876·0
,,	1901	42,790	45	444	1·04	950·9
,,	1902	404,430	476	11,688	2·89	849·6
,,	1903	No data.				
,,	1904	572,120	856	26,067	4·56	668·4
,,	1905	619,241	952	19,131	3·09	650·5
,,	1906	228,728	293	4125	1·80	780·6
,,	1907	598,384	892	15,707	2·63	670·8
Gujrat	1902	72,200	38	551	·76	1900·0
,,	1903	87,724	58	1794	2·05	1512·5
,,	1904	277,151	305	23,785	8·58	908·7
,,	1905	231,535	262	9782	4·22	883·7
,,	1906	120,219	60	1207	1·00	2000·7
,,	1907	560,579	853	51,000	9·10	657·2
Amritsar	1902	259,583	59	2039	·79	4399·7
,,	1903	700,040	480	27,285	3·90	1458·4
,,	1904	671,548	430	19,475	2·90	1561·7
,,	1905	846,148	644	30,719	3·63	1313·9
,,	1906	502,199	273	7911	1·58	1839·6
,,	1907	796,099	591	24,743	3·11	1347·0
Mozaffarnagar	1902	25,581	2	4	·02	12,790·5
,,	1903	73,944	23	782	1·06	3215·0
,,	1904	257,455	128	5486	2·13	2011·4
,,	1905	545,689	316	16,246	2·98	1726·9
,,	1906	174,409	68	3077	1·76	2564·8
,,	1907	753,403	590	49,909	6·62	1277·0
Rohtak	1904	147,918	56	2990	2·02	2641·4
,,	1905	491,746	284	28,802	5·86	1731·5
,,	1906	121,872	31	1948	1·60	3931·4
,,	1907	470,442	249	31,476	6·69	1889·3

NATIONAL ARCHIVES OF INDIA

TABLE XI.

AMRITSAR.

Epidemic of 1903.

Month of 1st infection	Total population	Number of deaths	Number of villages	Death rate per 1000	Mortality ratio March=1·00	Mean population of villages
Dec. '02	69,362	4324	52	62·34	1·09	1333·88
Jan.	71,890	3782	71	52·61	·92	1012·54
Feb.	84,558	5071	72	59·97	1·05	1174·42
March	104,639	5969	95	57·94	1·00	1101·46
April	89,723	4657	86	51·90	·91	1043·29
May	89,208	2533	86	28·39	·50	1037·30
June	9436	194	14	20·56	·36	674·00
	518,816	26,530	476	51·14	·883	1089·95

Epidemic of 1904.

Dec. '03	3879	56	2	14·44	·26	1939·5
Jan.	45,000	1715	20	38·11	·70	2250·0
Feb.	34,207	2103	23	61·48	1·12	1487·26
March	89,296	4886	70	54·72	1·00	1275·66
April	223,926	7980	207	35·64	·65	1081·77
May	92,137	1217	100	13·21	·24	921·37
June	1879	10	4	5·32	·01	469·75
	490,324	17,967	426	36·64	·670	1151·00

Epidemic of 1905.

Dec. '04	110,364	5826	75	52·79	1·13	1471·5
Jan.	120,386	5671	118	47·11	1·01	1020·2
Feb.	102,158	5153	95	50·44	1·08	1075·3
March	139,296	6510	128	46·74	1·00	1088·3
April	112,273	4551	125	40·54	·87	898·2
May	77,592	1729	93	22·28	·48	834·3
June	2855	38	6	13·31	·28	475·8
	664,924	29,478	640	44·33	·948	1038·94

NATIONAL ARCHIVES OF INDIA

TABLE XII.

AMRITSAR.

Epidemic of 1906.

Month of 1st infection	Population	No. of deaths	No. of villages	Death rate per 1000	Mortality ratio March=1·00	Mean population of villages
Dec. '05	2463	68	2	27·61	1·07	1231·5
Jan.	18,697	541	12	28·94	1·12	1558·1
Feb.	15,962	415	10	26·00	1·01	1596·2
March	64,953	1676	38	25·80	1·00	1709·3
April	97,009	2333	97	24·05	·93	1000·1
May	96,163	693	92	7·21	·28	1045·3
June	21,967	78	16	3·55	·14	1372·9
	317,214	5804	267	18·30	·709	1188·07

Epidemic of 1907.

Dec. '06	53,045	2701	42	50·92	1·20	1263·0
Jan.	35,877	1203	30	33·60	·79	1193·6
Feb.	60,723	2553	50	42·04	·99	1214·5
March	158,190	6732	128	42·56	1·00	1235·9
April	213,221	7528	197	35·31	·83	1082·3
May	103,421	2851	129	27·57	·65	801·7
June	17,965	260	27	14·47	·34	665·4
	642,442	23,828	603	37·09	·871	1065·4

NATIONAL ARCHIVES OF INDIA

TABLE XIII.

GUJRAT.

Epidemic of 1904.

Month of 1st infection	Total population	No. of deaths	No. of villages	Death rate per 1000	Mortality ratio March=1·00	Mean population of villages
Dec. '03	13,653	912	10	66·79	·42	1365·3
Jan.	3808	1051	4	276·00	1·75	952·0
Feb.	16,529	1790	14	108·29	·69	1180·6
March	58,132	9185	68	158·00	1·00	854·9
April	98,336	9719	143	98·83	·63	687·7
May	30,568	842	46	27·55	·17	664·5
June	12,327	144	16	11·68	·07	770·4
July	620	1	1	1·61	·01	620·0
	233,973	23,644	302	101·05	·64	774·8

Epidemic of 1905.

Dec. '04	11,359	821	14	72·28	1·31	811·4
Jan.	8998	594	16	66·01	1·20	562·4
Feb.	8685	629	13	72·42	1·31	668·1
March	48,480	2675	52	55·18	1·00	932·3
April	70,686	3697	93	52·30	·95	760·1
May	46,857	833	61	17·78	·32	768·2
June	5027	28	11	5·57	·10	457·0
July	—	—	—	—	—	—
	200,092	9277	260	46·36	·84	769·6

Epidemic of 1907.

Dec. '06	28,196	1741	16	61·75	·49	1762·3
Jan.	15,824	1909	20	120·63	·95	791·2
Feb.	40,836	5956	56	145·88	1·15	729·2
March	120,790	15,299	165	126·66	1·00	732·1
April	190,674	19,744	294	103·55	·82	648·6
May	102,997	4984	224	48·39	·38	459·8
June	26,014	563	67	21·64	·17	388·3
July	3805	39	9	10·25	·08	422·8
	529,136	50,235	851	94·94	·75	621·8

NATIONAL ARCHIVES OF INDIA

TABLE XIV.

HOSHIARPUR.

Epidemic of 1902.

Month of 1st infection	Total population	Number of deaths	Number of villages	Death rate per 1000	Mortality ratio (March=1·00)	Mean population of villages
Dec. '01	104,392	4543	116	43·52	3·07	899·9
Jan.	84,300	2720	89	32·27	2·28	947·2
Feb.	71,816	2342	98	32·61	2·30	732·8
March	67,473	957	79	14·18	1·00	854·1
April	47,918	896	52	18·70	1·32	921·5
May	22,864	176	34	7·70	·54	672·5
June	905	3	2	3·31	·23	452·5
July	4762	51	6	10·71	·76	793·7
	404,430	11,688	476	28·90	2·038	849·64

Epidemic of 1904.

Dec. '03	—	—	—	—	—	—
Jan.	62,900	2506	61	39·84	·81	1031·15
Feb.	96,191	5805	92	60·35	1·22	1045·55
March	110,282	5445	136	49·37	1·00	810·9
April	136,753	7047	249	51·53	1·04	549·1
May	127,600	4595	243	36·01	·73	525·1
June	30,176	446	62	14·78	·30	486·7
July	8218	223	13	27·14	·55	632·15
	572,120	26,067	856	45·56	·923	668·36

TABLE XV.

HOSHIARPUR.

Epidemic of 1905.

Month of 1st infection	Total population	Number of deaths	Number of villages	Death rate per 1000	Mortality ratio (March=1·00)	Mean population of villages
Dec. '04	71,162	3534	98	49·66	1·59	726·14
Jan.	194,770	6495	230	33·35	1·07	846·83
Feb.	87,999	2391	119	27·17	·87	739·49
March	82,962	2585	144	31·16	1·00	576·13
April	96,801	2640	186	27·27	·88	520·44
May	49,109	977	96	19·89	·64	511·55
June	29,774	423	65	14·21	·46	458·06
July	6664	86	14	12·91	·41	476·00
	619,241	19,131	952	30·89	·991	650·46

Epidemic of 1907.

Dec. '06	73,603	2475	99	33·63	1·14	743·46
Jan.	119,808	3346	123	27·93	·95	974·05
Feb.	96,984	3069	139	31·64	1·06	697·73
March	133,924	3937	224	29·40	1·00	597·88
April	113,387	2248	192	19·83	·67	590·56
May	39,691	569	90	14·34	·49	441·01
June	18,463	108	20	5·85	·20	923·15
July	2748	15	5	5·46	·19	549·60
	598,608	15,767	892	26·35	·893	671·10

NATIONAL ARCHIVES OF INDIA

TABLE XVI.

MOZAFFARNAGAR.

Epidemic of 1904.

Month of 1st infection	Total population	Number of deaths	Number of villages	Death rate per 1000	Mortality ratio March=1·00	Mean population of villages
Dec. '03	19,419	651	6	33·52	1·25	3236·5
Jan.	18,315	616	7	33·63	1·25	2616·4
Feb.	38,581	1331	20	34·50	1·28	1929·1
March	58,233	1567	30	26·91	1·00	1949·1
April	57,720	822	46	14·24	·53	1254·8
May	10,954	138	9	12·60	·47	1217·1
June	7663	24	7	3·13	·12	1094·7
July	—	—	—	—	—	—
	210,885	5149	125	24·42	·908	1687·08

Epidemic of 1905.

Dec. '04	137,988	4975	62	36·05	1·17	2225·6
Jan.	82,538	3012	48	36·49	1·18	1719·5
Feb.	39,781	1402	29	35·24	1·14	1371·8
March	126,554	3908	82	30·88	1·00	1543·34
April	67,114	2029	54	30·23	·98	1242·85
May	34,343	546	34	15·90	·51	1010·1
June	3069	7	4	2·28	·07	767·3
July	—	—	—	—	—	—
	491,387	15,879	313	32·31	1·046	1569·93

TABLE XVII.

MOZAFFARNAGAR.

Epidemic of 1906.

Month of 1st infection	Total population	Number of deaths	Number of villages	Death rate per 1000	Mortality ratio (March=1·00)	Mean population per village
Dec. '05	24,829	473	9	19·05	·66	2758·8
Jan.	3428	214	2	62·43	2·15	1714·0
Feb.	17,309	1014	8	58·58	2·02	2163·6
March	28,423	825	18	29·03	1·00	1579·1
April	27,346	298	19	10·90	·38	1439·3
May	15,895	85	8	5·35	·18	1986·9
June	2699	1	1	·37	·013	2699·0
July	—	—	—	—	—	—
	119,929	2910	65	24·26	·836	1845·06

Epidemic of 1907.

Dec. '06	83,580	3746	41	44·82	·503	2038·5
Jan.	87,134	7504	50	86·12	·95	1742·7
Feb.	133,234	10,522	81	78·97	·88	1644·9
March	145,205	13,126	133	90·40	1·00	1091·8
April	160,761	9665	174	60·12	·67	923·9
May	80,158	1952	99	24·35	·27	809·7
June	9022	122	10	13·52	·15	902·2
July	—	—	—	—	—	—
	699,094	46,637	588	66·77	·739	1188·94

NATIONAL ARCHIVES OF INDIA

TABLE XVIII.

ROHTAK.

Epidemic of 1905.

Month of 1st infection	Total population	Number of deaths	Number of villages	Death rate per 1000	Mortality ratio (March=1·00)	Mean population of villages
Dec. '04	63,620	3500	25	55·01	·64	2544·8
Jan.	97,979	7387	41	75·39	·88	2389·7
Feb.	67,554	4588	43	67·92	·79	1571·0
March	82,796	7080	57	85·51	1·00	1452·6
April	114,258	4687	95	41·02	·48	1202·7
May	30,624	401	19	13·09	·15	1611·8
June	1095	8	1	7·31	·09	1095·0
Total	457,926	27,651	281	60·38	·71	1629·6

Epidemic of 1907.

Dec. '06	38,129	3603	11	94·50	1·16	3466·3
Jan.	29,508	3141	11	106·40	1·58	2228·0
Feb.	57,078	4400	26	77·09	·96	2195·3
March	169,885	13,799	85	81·23	1·00	1998·7
April	96,351	5220	73	54·18	·67	1319·9
May	42,900	643	35	14·99	·18	1225·7
June	4290	43	6	10·02	·12	715·0
Total	438,141	30,849	247	70·40	·88	1753·6

TABLE XVIII A.

Particulars of villages infected in December or earlier and grouped together in Tables XI—XVIII.

HOSHIARPUR 1907.

Month of 1st infection	Population	Deaths	No. of villages	Mortality %	Average population of villages
July	7010	283	7	4·04	905·8
August	434	20	3	4·61	144·7
September	764	3	2	·39	382·0
October	4803	142	8	2·96	600·4
November	28,136	832	35	2·96	803·9
December	32,456	1195	44	3·68	737·6
Total	73,603	2475	99	3·36	743·5

AMRITSAR 1907.

September	1409	72	2	5·11	704·5
October	3469	121	3	3·49	1156·3
November	17,175	1039	14	6·05	1226·8
December	30,992	1469	23	4·74	1347·5
Total	53,045	2701	42	5·09	1263·0

MOZAFFARNAGAR 1907.

October	12,266	515	7	4·20	1752·3
November	21,436	799	8	3·73	2679·5
December	49,878	2432	26	4·88	1918·4
Total	83,580	3746	41	4·48	2036·6

NATIONAL ARCHIVES OF INDIA

TABLE XIX.

Relation between length of exposure and number of deaths in the whole epidemic.

Population group	Number of villages	Number of deaths	Mean deaths per village	Population	Mean population of villages	Standard deviation of population	Coefficient of variation	r
Hoshiarpur 1902:								
10— 200	46	380	8·3	5416	117·7	56·5	47·98	+·44±·08
130— 270	57	576	10·1	11,763	206·4	40·2	19·47	+·41±·07
250— 350	57	565	9·9	17,067	299·4	28·6	9·56	+·37±·08
350— 450	56	683	12·2	22,099	394·6	29·0	7·36	+·40±·08
450— 550	41	797	19·4	20,365	496·7	28·5	5·73	+·29±·10
525— 675	58	987	17·0	34,641	597·3	42·4	7·10	+·31±·08
625— 775	41	905	22·1	28,626	698·2	50·2	7·19	+·47±·08
700— 900	51	1318	25·8	41,009	804·1	55·7	6·92	+·46±·07
800—1000	48	1544	32·2	43,073	897·4	61·3	6·83	+·29±·09
875—1125	50	1676	33·5	49,710	994·2	70·5	7·09	+·38±·08
Hoshiarpur 1905:								
75— 125	48	357	7·4	4765	99·3	16·0	16·07	−·06±·10
125— 175	62	481	7·8	9228	148·8	15·0	10·09	+·41±·07
175— 225	73	671	9·2	14,818	203·0	14·5	7·14	+·36±·07
225— 275	75	776	10·3	18,642	248·6	15·7	6·32	+·21±·07
275— 325	65	494	7·6	19,438	299·0	14·7	4·91	+·14±·08
325— 375	76	1135	14·9	26,799	352·6	15·0	4·24	+·34±·07
375— 425	50	636	12·7	20,001	400·0	15·2	3·79	+·18±·09
425— 475	61	837	13·7	27,186	445·7	15·1	3·40	+·48±·07
475— 525	41	639	15·6	20,529	500·7	14·8	2·95	+·43±·09
520— 580	45	750	16·7	24,846	552·1	18·7	3·39	+·26±·09
550— 650	75	1369	18·3	44,972	599·6	29·0	4·84	+·29±·07
650— 750	41	680	16·6	28,747	701·1	28·5	4·06	+·33±·09
750— 850	47	1138	24·2	37,658	801·2	30·4	3·79	+·38±·08
840— 960	40	970	24·3	35,957	898·9	35·6	3·96	+·40±·09
925—1075	48	1374	28·6	47,846	996·8	46·1	4·63	+·38±·08
1000—1200	42	1215	28·9	45,617	1086·1	53·9	4·97	+·17±·10
1050—1350	49	1771	36·1	57,901	1181·7	92·3	7·81	+·35±·08
1150—1450	42	1739	41·4	54,940	1308·1	80·3	6·14	+·28±·10
1200—1600	50	2489	49·8	69,018	1380·4	99·9	7·24	+·24±·09
1300—1700	45	2316	51·5	65,412	1453·6	107·6	7·40	+·11±·10
1400—1800	38	1997	52·6	60,092	1581·4	127·5	8·06	+·28±·10
Hoshiarpur 1907:								
75— 125	50	664	13·3	4984	99·7	14·1	14·12	+·18±·09
175— 225	62	681	11·0	12,395	199·9	15·9	7·93	+·37±·07
275— 325	51	638	12·5	15,178	297·6	14·5	4·87	+·14±·09
370— 430	60	655	10·9	24,135	402·3	19·9	4·94	+·28±·07
450— 550	70	1046	14·9	34,631	494·7	28·8	5·82	+·27±·07
550— 650	67	1229	18·3	40,143	603·2	31·2	5·17	+·60±·05
650— 750	40	720	18·0	27,839	695·4	31·8	4·58	+·12±·11
725— 875	64	1203	18·8	51,111	798·6	42·3	5·30	+·25±·08
825— 975	57	1234	21·6	51,243	899·0	47·4	5·27	+·48±·07
925—1075	46	1100	23·9	46,029	1000·6	47·3	4·73	+·34±·09
990—1210	42	1243	29·6	45,282	1078·1	56·3	5·23	+·20±·10
1025—1375	55	1440	26·2	64,837	1178·9	117·7	9·98	+·37±·08
1125—1475	43	1212	28·2	56,538	1314·8	96·7	7·36	+·50±·08
1200—1600	48	1358	28·3	67,108	1398·1	97·4	6·96	+·40±·08
1300—1700	49	1588	32·4	71,416	1457·5	107·6	7·38	+·47±·08
1400—1800	40	1285	32·1	63,344	1583·6	122·1	7·71	+·59±·07
Amritsar 1903:								
100— 300	31	845	27·3	6864	221·4	50·1	22·63	+·09±·12
400— 500	45	1333	29·6	20,528	456·2	28·2	6·18	−·02±·10

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TABLE XIX (*continued*).

Population group	Number of villages	Number of deaths	Mean deaths per village	Population	Mean population of villages	Standard deviation of population	Coefficient of variation	r
Amritsar 1903 (*continued*):								
700— 900	72	2849	39·6	57,254	795·2	53·5	6·70	+·13±·08
900—1100	48	2792	58·2	47,709	993·9	64·9	6·53	+·00±·10
1050—1350	56	3254	58·1	66,495	1187·4	90·0	7·58	+·35±·08
1150—1450	47	3182	67·7	60,434	1285·8	85·6	6·66	+·21±·09
1250—1550	48	3354	69·9	67,270	1401·5	85·2	6·08	+·20±·09
1350—1650	43	2916	67·8	64,428	1498·3	78·7	5·25	+·22±·10
1450—1750	43	3094	72·0	67,986	1581·1	91·9	5·81	+·45±·08
1700—2300	45	3776	83·9	86,493	1922·1	146·5	7·62	+·33±·09
1850—3150	43	4133	96·1	96,943	2254·5	374·1	16·59	+·35±·09
2000—4000	40	4750	118·8	108,251	2706·3	572·7	21·16	+·49±·08
Amritsar 1904:								
100— 300	42	610	14·5	9536	227·0	48·7	21·44	+·41±·09
400— 500	25	477	19·1	11,240	449·6	28·3	6·28	−·38±·12
700— 900	56	1259	22·5	44,196	789·2	54·0	6·84	+·11±·09
900—1100	41	2051	50·0	40,785	994·8	56·5	5·68	+·48±·08
1050—1350	50	2522	50·4	59,898	1198·0	89·1	7·44	+·58±·06
1150—1450	42	2181	51·9	53,703	1278·6	81·5	6·38	+·46±·08
1250—1550	43	2467	57·4	59,972	1394·7	89·7	6·43	+·35±·09
1350—1650	40	2849	71·2	60,091	1502·3	84·9	5·65	+·39±·09
1450—1750	44	2743	62·3	69,952	1589·8	89·9	5·65	+·25±·10
1700—2300	42	2853	67·9	81,198	1933·3	155·4	8·04	+·39±·09
1900—3100	39	2543	65·2	90,782	2327·7	334·8	14·38	+·13±·11
2000—4000	40	2901	72·5	105,171	2629·3	530·2	20·17	+·10±·11
Amritsar 1905:								
100— 300	60	1290	21·5	13,668	227·8	46·9	20·57	+·20±·08
400— 500	55	1329	25·2	25,005	454·6	27·9	6·15	+·42±·07
700— 900	91	3591	39·5	72,668	798·5	54·4	6·81	+·33±·06
900—1100	60	3301	55·0	59,318	988·6	64·5	6·52	+·35±·08
1100—1300	45	2313	51·4	53,814	1195·9	58·3	4·87	+·53±·07
1200—1400	41	2040	49·8	53,165	1296·7	60·4	4·66	+·18±·10
1250—1550	58	3345	57·7	81,257	1401·0	83·5	5·96	+·17±·09
1350—1650	57	3410	59·8	84,968	1490·7	85·1	5·71	+·18±·09
1450—1750	52	2961	56·9	80,789	1553·6	74·8	4·82	+·34±·08
1700—2300	51	3438	67·4	98,925	1939·7	158·2	8·16	+·13±·09
1900—3100	46	4020	87·4	106,765	2321·0	329·7	14·21	+·27±·09
2000—4000	53	4904	92·5	138,576	2614·6	549·3	21·01	+·16±·09
Amritsar 1907:								
100— 300	60	1266	21·1	13,284	221·4	52·5	23·73	+·31±·08
400— 500	52	1258	24·2	23,393	449·9	26·9	5·98	+·30±·09
700— 900	82	3000	36·6	65,685	801·0	55·1	6·87	+·29±·07
900—1100	57	2136	37·5	56,481	990·9	64·2	6·48	+·33±·08
1100—1300	45	1825	40·6	53,932	1198·5	56·9	4·75	+·28±·09
1150—1450	55	2408	43·8	70,383	1328·0	82·2	6·19	+·25±·09
1250—1550	49	2405	49·1	68,259	1393·0	86·0	6·17	+·41±·08
1350—1650	44	2284	51·9	65,672	1492·5	83·4	5·59	+·42±·08
1450—1750	44	2086	47·4	69,781	1585·9	86·8	5·47	+·41±·08
1700—2300	53	3106	58·6	102,469	1933·4	147·9	7·65	+·26±·09
1900—3100	47	3594	76·5	108,123	2300·5	329·0	14·30	+·19±·09
2000—4000	50	4081	81·6	131,778	2635·6	539·3	20·46	+·24±·09
Gujrat 1907:								
400— 500	83	3914	47·2	37,385	450·4	28·0	6·22	+·35±·06
750— 850	41	3432	83·7	32,650	796·3	30·7	3·86	+·21±·10
900—1100	52	4777	91·9	52,338	1006·5	62·2	6·18	+·29±·09
1050—1350	47	4945	105·2	54,167	1152·5	70·5	6·12	+·15±·10

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TABLE XX.

Mean values of coefficients in Table XIX.

District	Year	Mean r	S.D.	Coeff. of var.
Amritsar	1903	·23	·154	66·96
	1904	·33	·221	66·97
	1905	·27	·113	41·85
	1907	·30	·066	22·00
Hoshiarpur	1902	·38	·062	16·32
	1905	·29	·122	42·07
	1907	·35	·144	41·14
Gujrat	1907	·27	·079	29·26

TABLE XXI.

Correlation between length of exposure and total number of plague deaths for a constant population.

(Villages up to 3000 inhabitants.)

District	Epidemic	No. of villages	Coeff. of correlation
Hoshiarpur	1901—2	447	·25 ± ·03
,,	1903—4	844	·24 ± ·02
,,	1904—5	950	·24 ± ·02
,,	1905—6	284	·29 ± ·04
,,	1906—7	875	·25 ± ·02
Gujrat	1903—4	296	·39 ± ·03
,,	1904—5	257	·27 ± ·04
,,	1906—7	843	·28 ± ·02
Rohtak	1904—5	232	·30 ± ·04
,,	1906—7	200	·30 ± ·04
Amritsar	1902—3	456	·19 ± ·03
,,	1903—4	412	·34 ± ·03
,,	1904—5	643	·29 ± ·02
,,	1906—7	560	·28 ± ·03
Mozaffarnagar	1904—5	266	·05 ± ·04
,,	1906—7	536	·27 ± ·03

Mean values for different districts.

Hoshiarpur	·25	Amritsar	·28
Gujrat	·30	Mozaffarnagar	·20
Rohtak	·30		

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TABLE XXI A.

Amritsar (1902–3). *Month of first infection.*

Population group	December	January	February	March	April	May	June	Frequency
0— 200	1·0	1·0	2·0	3·0	4·0	4·0	—	15·0
200— 400	3·0	10·0	5·0	2·0	12·0	14·0	5·0	51·0
400— 600	4·0	13·0	14·0	23·0	8·0	17·0	2·0	81·0
600— 800	4·0	12·0	12·0	16·0	16·0	12·0	4·0	76·0
800—1000	10·0	6·0	8·0	14·0	12·0	8·0	1·0	59·0
1000—1200	7·0	6·0	9·0	6·0	8·0	3·0	1·0	40·0
1200—1400	7·0	8·0	2·5	7·0	3·0	7·0	—	34·5
1400—1600	1·0	1·0	2·5	6·0	10·0	10·0	—	30·5
1600—1800	4·0	5·0	3·0	6·0	3·0	3·0	—	24·0
1800—2000	6·0	4·0	5·0	1·0	2·0	2·0	—	20·0
2000—2200	—	1·0	2·0	4·0	1·0	4·0	1·0	13·0
2200—2400	1·0	—	—	1·0	2·0	—	—	4·0
2400—2600	—	1·0	—	1·0	1·0	1·0	—	4·0
2600—2800	—	—	—	—	1·0	—	—	1·0
2800—3000	—	1·0	1·0	1·0	—	—	—	3·0
Total	48·0	69·0	66·0	91·0	83·0	85·0	14·0	456·0

TABLE XXI B.

Amritsar (1902–3). *Population groups.*

Number of deaths	0— 200	200— 400	400— 600	600— 800	800—1000	1000—1200	1200—1400	1400—1600	1600—1800	1800—2000	2000—2200	2200—2400	2400—2600	2600—2800	2800—3000	Frequency
1— 5	3·0	2·0	4·0	2·0	3·0	2·0	2·0	1·0	—	—	1·0	—	—	—	—	20·0
6— 15	2·0	15·0	16·5	14·0	8·0	5·5	2·0	3·0	—	1·5	1·0	—	—	—	—	68·5
15— 30	8·0	18·0	25·5	19·0	10·0	5·5	5·0	5·5	4·0	3·0	2·0	—	—	—	—	105·5
30— 45	1·0	5·0	18·0	15·0	8·5	3·5	7·5	4·0	2·5	1·5	1·0	—	1·0	—	—	68·5
45— 60	1·0	3·0	7·5	15·5	12·0	6·5	5·0	6·0	6·5	·5	1·5	1·0	·5	—	—	66·5
60— 75	—	3·5	4·5	4·5	5·5	6·5	3·0	—	—	4·5	·5	—	·5	—	—	33·0
75— 90	—	·5	1·5	1·0	5·5	5·0	1·5	2·0	1·5	2·5	1·0	1·0	—	—	—	23·0
90—105	—	1·5	2·5	4·0	4·0	2·0	3·5	5·0	3·5	3·5	4·0	1·0	—	—	—	34·5
105—120	—	·5	—	—	1·0	1·5	—	1·0	2·0	—	—	—	—	—	—	6·0
120—135	—	—	—	1·0	·5	—	—	—	—	—	1·0	—	—	—	2·0	4·5
135—150	—	1·0	1·0	—	—	—	·5	1·0	—	—	—	—	·5	—	1·0	5·0
150—165	—	1·0	—	—	—	—	·5	—	—	—	—	—	·5	1·0	—	3·0
165—180	—	—	—	—	—	·5	·5	—	1·0	—	—	—	1·0	—	—	3·0
180—195	—	—	—	—	—	·5	·5	1·0	1·5	—	—	·5	—	—	—	4·0
195—210	—	—	—	—	—	1·0	1·0	—	·5	—	—	·5	—	—	—	3·0
210—225	—	—	—	—	1·0	—	—	—	—	1·0	—	—	—	—	—	2·0
225—240	—	—	—	—	—	—	1·0	—	—	—	—	—	—	—	—	1·0
240—255	—	—	—	—	—	—	·5	1·0	1·0	—	—	—	—	—	—	2·5
255—270	—	—	—	—	—	—	·5	—	—	1·0	—	—	—	—	—	1·5
270—285	—	—	—	—	—	—	—	—	—	1·0	—	—	—	—	—	1·0
Total	15·0	51·0	81·0	76·0	59·0	40·0	34·5	30·5	24·0	20·0	13·0	4·0	4·0	1·0	3·0	456·0

NATIONAL ARCHIVES OF INDIA

TABLE XXI C.

Amritsar (1902–3). *Month of first infection.*

Number of deaths	December	January	February	March	April	May	June	Frequency
1— 5	4·0	5·0	—	2·0	2·0	6·0	1·0	20·0
6— 15	5·5	13·0	8·5	9·0	6·0	18·0	8·5	68·5
15— 30	12·0	17·0	5·5	16·5	22·0	28·0	3·5	104·5
30— 45	3·5	7·0	13·5	15·5	10·5	18·5	1·0	69·5
45— 60	4·0	6·5	11·5	15·5	18·5	10·5	—	66·5
60— 75	2·0	7·5	6·0	8·0	8·5	—	—	32·0
75— 90	4·0	2·0	6·5	5·0	3·5	2·0	—	23·0
90—105	3·5	4·0	11·0	9·5	6·0	·5	—	34·5
105—120	3·5	—	·5	·5	1·0	·5	—	6·0
120—135	—	1·0	—	3·5	1·0	—	—	5·5
135—150	—	1·5	1·0	1·5	—	1·0	—	5·0
150—165	—	1·5	—	·5	1·0	—	—	3·0
165—180	—	2·0	—	·5	·5	—	—	3·0
180—195	—	1·0	—	2·5	·5	—	—	4·0
195—210	2·0	—	—	1·0	—	—	—	3·0
210—225	1·0	—	—	—	1·0	—	—	2·0
225—240	—	—	1·0	—	—	—	—	1·0
240—255	1·5	—	—	—	1·0	—	—	2·5
255—270	·5	—	1·0	—	—	—	—	1·5
270—285	1·0	—	—	—	—	—	—	1·0
Total	48·0	69·0	66·0	91·0	83·0	85·0	14·0	456·0

TABLE XXII.

Average duration of plague in villages infected in different months.

Month of 1st infection	No. of villages first reporting deaths in this month		Average No. of months in which deaths continued to be reported	
	Gujrat 1906—7	Hoshiarpur 1906—7	Gujrat 1906—7	Hoshiarpur 1906—7
December	16	97	5·44	2·96
January	20	122	4·45	2·98
February	56	143	3·91	2·69
March	165	224	3·25	2·18
April	294	192	2·67	1·63
May	223	90	2·04	1·28
June	67	20	1·28	1·10
July	9	4	1·00	1·00

TABLE XXII A.

Death rates of villages reporting deaths in certain sequences.

District and year	Sequence January, February, March			Sequence February, March, April		
	Population	Deaths	Rate	Population	Deaths	Rate
Mozaffarnagar 1907	7135	621	8·70	23,072	1532	6·64
Amritsar 1907	7017	345	4·92	8385	397	4·73
Amritsar 1905	18,177	743	4·09	30,930	1272	4·11
Hoshiarpur 1905	25,424	894	3·52	21,878	658	3·01
Hoshiarpur 1907	15,317	621	4·05	21,757	966	4·44
Total	73,070	3224	4·41	106,022	4825	4·55

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TABLE XXIII.

Particulars of epidemics in which over 300 villages were infected in any district.

District and year	Particulars for all villages infected					Infected villages over 3000 population					Infected villages under 3000 population				
	Total population	Number of villages	Total mortality	Death rate per cent.	Average population of villages	Total population	Number of villages	Total mortality	Death rate per cent.	Average population of villages	Total population	Number of villages	Total mortality	Death rate per cent.	Average population of villages
Hoshiarpur:															
1902	404,430	476	11,688	2·89	849·6	63,755	12	864	1·36	5312·9	340,675	464	10,824	3·18	734·2
1904	572,120	856	26,067	4·56	668·4	65,952	12	1425	2·16	5496·0	506,168	844	24,642	4·87	599·7
1905	619,241	952	19,131	3·09	650·5	63,305	12	1052	1·66	5275·4	555,936	940	18,079	3·25	591·4
1907	598,384	892	15,707	2·63	670·8	68,079	13	962	1·41	5236·8	530,305	879	14,745	2·78	603·3
Amritsar:															
1903	537,611	479	26,825	4·99	1122·4	104,317	25·5	3839	3·68	4090·8	433,294	453·5	22,986	5·30	955·4
1904	509,119	429	18,372	3·61	1186·8	112,456	26·0	3184	2·83	4325·2	396,663	403·0	15,188	3·83	984·3
1905	683,719	643	29,646	4·34	1063·3	130,630	31·5	4263	3·26	4147·0	553,089	611·5	25,383	4·59	904·5
1907	633,670	590	23,642	3·73	1074·0	120,547	28·5	3010	2·50	4229·7	513,123	561·5	20,632	4·02	913·8
Gujrat:															
1904	277,151	305	23,785	8·58	908·7	69,671	9	849	1·22	7741·2	207,480	296	22,936	11·05	700·9
1907	560,579	853	51,000	9·10	657·2	68,231	10	2718	3·98	6823·1	492,348	843	48,282	9·81	584·0
Mozaffarnagar:															
1905	545,689	316	16,246	2·98	1726·9	247,026	43	6961	2·82	5744·8	298,663	273	9285	3·11	1094·0
1907	753,403	590	49,909	6·62	1277·0	264,202	49	16,365	6·19	5391·9	489,201	541	33,544	6·86	904·3

NATIONAL ARCHIVES OF INDIA

TABLE XXIV.

Particulars of epidemics in which less than 300 villages were infected in any district.

District and year	Particulars for all villages infected					Infected villages over 3000 population					Infected villages under 3000 population				
	Total population	Number of villages	Total mortality	Death rate per cent.	Average population of villages	Total population	Number of villages	Total mortality	Death rate per cent.	Average population of villages	Total population	Number of villages	Total mortality	Death rate per cent.	Average population of villages
Hoshiarpur:															
1898	17,438	16	439	2·52	1089·9	5803	1	87	1·50	5803·0	11,635	15	352	3·03	775·7
1899	3701	4	9	·24	925·3	—	—	—	—	—	3701	4	9	·24	925·3
1900	9636	11	50	·52	876·0	—	—	—	—	—	9636	11	50	·52	876·0
1901	42,790	45	444	1·04	950·9	9030	2	112	1·24	4515·0	33,760	43	332	·98	785·1
1903?	No data	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1906	228,728	293	4125	1·80	780·6	52,038	9	612	1·18	5782·0	176,690	284	3513	1·99	622·1
Gujrat:															
1902	72,200	38	551	·76	1900·0	37,875	3	14	·04	12,625·0	34,325	35	537	1·56	980·7
1903	87,724	58	1794	2·05	1512·5	50,902	6	673	1·32	8483·7	36,822	52	1121	3·04	708·1
1905	231,535	262	9782	4·22	883·7	46,619	5	775	1·66	9323·8	184,916	257	9007	4·87	719·5
1906	120,219	60	1207	1·00	2000·7	81,328	8	40	·05	10,166·0	38,891	52	1167	3·00	747·9
Mozaffarnagar:															
1902	25,581	2	4	·02	12,790·5	23,444	1	1	·00	23,444·0	2137	1	3	·14	2137·0
1903	73,944	23	782	1·06	3215·0	50,509	5	257	·51	10,101·8	23,435	18	525	2·24	1301·9
1904	257,455	128	5486	2·13	2011·4	131,227	20	2371	1·81	6561·4	126,228	108	3115	2·47	1168·8
1906	174,409	68	3077	1·76	2564·8	103,924	14	1526	1·47	7423·1	70,485	54	1551	2·20	1305·3
Rohtak:															
1904	147,918	56	2990	2·02	2641·4	108,513	17	1996	1·84	6383·1	39,405	39	994	2·52	1010·4
1905	491,746	284	28,802	5·86	1731·5	229,747	46	12,792	5·57	4994·5	261,999	238	16,010	6·11	1100·8
1906	121,872	31	1948	1·60	3931·4	97,761	16	1275	1·30	6110·1	24,111	15	673	2·79	1607·4
1907	469,355	247	31,330	6·68	1900·2	222,249	44	14,619	6·58	5096·6	247,106	203	16,711	6·76	1217·3
Amritsar:															
1902	97,154	58	1957	2·01	1675·1	34,514	7	651	1·89	4930·6	62,640	51	1306	2·08	1228·2
1906	339,770	272	6008	1·77	1249·2	90,985	21·5	1217	1·34	4231·9	248,785	250·5	4791	1·93	993·2

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TABLE XXV.

Plague experience in towns of over 13,500 *inhabitants.*

Name of town	Population	Number of deaths	Mortality per cent.	Month of 1st infection	Number of months epidemic continued
Rohtak Town:					
1904	20,024	13	·065	February	4
1905	20,024	888	4·435	Oct. '04	8
1906	20,024	3	·015	April	2
1907	20,024	464	2·317	February	5
Amritsar City:					
1902	162,429	82	·051	February	Continuously infected.
1903	162,429	460	·283	Continuous	19
1904	162,429	1103	·679	January	7
1905	162,429	1073	·661	January	20
1906	162,429	1903	1·172	Continuous	
1907	162,429	1101	·678	January	7
Mozaffarnagar:					
1902	23,444	1	·004	January	1
1903	23,444	25	·107	February	5
1904	23,444	331	1·412	Oct. '03	7
1905	23,444	249	1·062	Nov. '04	7
1906	23,444	112	·478	March	4
* 1907	23,444	490	2·090	Nov. '06	7
Kairana (Mozaffarnagar District):					
1905	19,304	94	·487	January	6
1906	19,304	2	·010	April	1
1907	19,304	2136	11·065	February	5
Gujrat:					
1902	19,708	11	·056	May	2
1903	19,708	281	1·426	Aug. '02	12
1904	19,708	89	·452	Oct. '03	10
1905	19,708	183	·929	Oct. '04	9
1906	19,708	18	·091	April	2
1907	19,708	237	1·203	Oct. '06	10

* One further case occurred here in Sept. 1907, three months after epidemic ceased.

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TABLE XXVI.

HOSHIARPUR 1907.

Infected villages having less than 1000 *population, showing month of* 1*st infection.*

Month of 1st infection	Population	Number of villages	Number of deaths	Mortality per cent.	Average population of villages
Dec. '06	32,914	74	1277	3·88	444·8
January	36,667	87	1529	4·17	421·5
February	53,767	117	2204	4·10	459·5
March	71,666	185	2681	3·74	387·4
April	65,994	167	1585	2·40	395·2
May	32,643	85	524	1·61	384·0
June	7486	16	86	1·15	467·9
July	1535	4	14	·91	383·8
Total	302,672	735	9900	3·27	411·8

Infected villages having population over 1000 *and under* 3000, *showing month of* 1*st infection.*

Dec. '06	40,689	25	1198	2·94	1627·6
January	43,073	29	1054	2·45	1485·3
February	28,723	19	865	3·01	1511·7
March	62,258	39	1256	2·02	1596·4
April	40,908	24	663	1·62	1704·5
May	7048	5	45	·64	1409·6
June	3945	2	13	·33	1972·5
July	1213	1	1	·08	1213·0
Total	227,857	144	5095	2·24	1582·3

TABLE XXVII.

AMRITSAR 1907.

Infected villages having less than 1000 *population, with month of* 1*st infection.*

Month of 1st infection	Population	Number of villages	Number of deaths	Mortality per cent.	Average population of villages
Dec. '06	13,900	22	1011	7·27	631·8
January	9557	17	496	5·19	562·2
February	12,569	23	715	5·69	546·5
March	41,537	73	2837	6·83	569·0
April	66,540	122	3535	5·31	545·4
May	52,056	95	1793	3·44	548·0
June	9087	21	173	1·90	432·7
Total	205,246	373	10,560	5·15	550·3

Villages with population from 1000—3000, *and month of* 1*st infection.*

Dec. '06	26,407	17	1124	4·26	1553·4
January	12,699	9	482	3·80	1411·0
February	40,448	25	1481	3·66	1617·9
March	78,808	46	2816	3·57	1713·2
April	102,760	65	3406	3·31	1580·9
May	48,149	33	974	2·02	1459·1
June	8878	6	87	·98	1479·7
Total	318,149	201	10,370	3·26	1582·8

NATIONAL ARCHIVES OF INDIA

TABLE XXVIII.

MOZAFFARNAGAR 1907.

Infected villages having less than 1000 *population, showing month of* 1*st infection.*

Month of 1st infection	Population	Number of villages	Number of deaths	Mortality per cent.	Average population of villages
Dec. '06	10,292	16	375	3·64	643·3
January	9839	16	887	9·02	614·9
February	20,686	39	2315	11·19	530·4
March	42,767	77	4365	10·21	555·4
April	58,164	118	4026	6·92	492·9
May	35,079	68	1176	3·35	515·9
June	3304	7	68	2·06	472·0
Total	180,131	341	13,212	7·33	528·2

Infected villages with population from 1000 *to* 3000, *showing month of* 1*st infection.*

Dec. '06	32,425	18	1727	5·33	1801·4
January	41,076	25	3594	8·75	1643·0
February	41,792	27	3574	8·55	1547·9
March	77,593	48	6201	7·99	1616·5
April	71,259	47	4123	5·79	1516·1
May	38,740	26	631	1·63	1490·0
June	4102	3	56	1·37	1367·3
Total	306,987	194	19,906	6·48	1582·4

TABLE XXIX.

GUJRAT 1907.

Infected villages with population up to 1000, *showing month of* 1*st infection.*

Month of 1st infection	Population	Number of villages	Number of deaths	Mortality per cent.	Average population of villages
Dec. '06	2494	5	264	10·59	498·8
January	8509	16	1252	14·71	531·8
February	18,364	43	2894	15·76	427·1
March	64,209	132	9144	14·24	486·4
April	103,341	243	12,726	12·31	425·3
May	76,499	203	4350	5·69	376·8
June	20,552	64	509	2·48	321·1
July	2402	8	34	1·42	300·3
Total	296,370	714	31,173	10·52	405·1

Infected villages from 1000 *to* 3000 *population, with month of* 1*st infection.*

Dec. '06	14,002	9	887	6·33	1555·8
January	7315	4	657	8·98	1828·8
February	19,353	12	2806	14·50	1612·8
March	47,987	31	5275	10·99	1548·0
April	73,958	48	6780	9·17	1540·8
May	26,498	21	634	2·39	1261·8
June	5462	3	54	·99	1820·7
July	1403	1	5	·36	1403·0
Total	195,978	129	17,098	8·72	1519·2

NATIONAL ARCHIVES OF INDIA

TABLE XXX.

ROHTAK 1907.

Infected villages having less than 1000 *population, showing month of* 1*st infection.*

Month of 1st infection	Population	Number of villages	Number of deaths	Mortality per cent.	Average population of villages
Dec. '06	—	—	—	—	—
January	1082	2	53	4·90	541·0
February	6428	12	706	10·98	535·7
March	12,592	21	1050	8·34	599·6
April	17,688	31	1077	6·09	570·6
May	13,565	20	318	2·34	678·3
June	3091	5	20	·65	618·2
Total	54,446	91	3224	5·92	598·3

Infected villages with populations from 1000 *to* 3000, *showing month of* 1*st infection.*

Dec. '06	10,257	5	631	6·15	2051·4
January	4707	4	416	8·84	1176·8
February	7089	5	628	8·86	1417·8
March	85,310	47	8011	9·39	1815·1
April	64,589	38	3478	5·38	1699·7
May	19,348	12	300	1·55	1612·3
June	1360	1	23	1·69	1360·0
Total	192,660	112	13,487	7·00	1720·2

NATIONAL ARCHIVES OF INDIA

TABLE XXXI.

Comparison of mortality in villages with population of under 300 *with the mortality in those having a population of* 300 *or over in certain districts and epidemics.*

District and year	Infected first in month of	Total population	Number of villages	Total number of deaths	Mortality per cent.	Average population of villages
Population under 300.						
Gujrat 1907	April	17,627	92	2673	15·16	191·60
	May	16,445	88	1178	7·16	186·88
Hoshiarpur 1904	April	15,967	88	1429	8·95	181·44
	May	16,889	95	1013	6·00	177·78
Hoshiarpur 1907	March	15,099	82	892	5·91	184·13
	April	12,436	67	567	4·56	185·61
Population 300 or over.						
Gujrat 1907	April	173,047	202	17,071	9·86	856·67
	May	86,552	136	3806	4·40	636·41
Hoshiarpur 1904	April	120,786	161	5618	4·65	750·22
	May	110,711	148	3582	3·24	748·05
Hoshiarpur 1907	March	118,825	162	3045	2·56	733·49
	April	100,951	125	1681	1·67	807·61
All infected villages.						
Gujrat 1907	April	190,674	294	19,744	10·36	648·55
	May	102,997	224	4984	4·84	459·80
Hoshiarpur 1904	April	136,753	249	7047	5·15	549·10
	May	127,600	243	4595	3·60	525·10
Hoshiarpur 1907	March	133,924	224	3937	2·94	597·88
	April	113,387	192	2248	1·98	590·56

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TABLE XXXII.

ROHTAK 1907.

Tehsil	Villages with population below 1000			Villages with population between 1000—3000		
	Total population	Deaths	Percentage	Total population	Deaths	Percentage
Rohtak	13,794	774	5·6	39,529	1547	3·9
Gohannah	11,434	686	6·0	69,668	5072	7·3
Sampla	20,052	1374	6·9	73,518	6665	9·1
Jhajjar	9166	390	4·3	9945	203	2·0

TABLE XXXIII.

Proportions of villages in different groups infected in 1907*.

District	Mean population of villages 0—1000	Mean population of villages 1000—3000	Percentage No. of villages 0—1000 infected 1906—7	Percentage No. of villages 1000—3000 infected 1906—7	Ratio of percentage infections for the two groups	Ratio of mean populations for the two groups
Rohtak	458·5	1655·7	30·4	73·7	2·4	3·6
Amritsar	457·8	1539·3	47·7	81·7	1·7	3·4
Mozaffarnagar	413·4	1584·6	48·8	86·2	1·8	3·8
Hoshiarpur	334·5	1525·0	39·3	67·3	1·7	4·6

* Gujrat not included owing to uncertainties respecting populations of non-infected villages.

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TABLE XXXIII A.

Particulars of villages in the various districts which were not infected in 1907.

District	Villages with population up to 1000			Population 1000—3000			Population over 3000			Total		
	Population	No. of villages	Average population	Population	No. of villages	Average population	Population	No. of villages	Average population	Population	No. of villages	Average Population
Rohtak :												
Never infected	48,781	136	358·7	14,270	9	1585·6	—	—	—	63,051	145	434·8
Not infected 1907	33,853	72	470·2	44,732	31	1443·0	7498	2	3749·0	86,083	105	819·8
Total	82,634	208	397·3	59,002	40	1475·0	7498	2	3749·0	149,134	250	596·5
Amritsar :												
Never infected	34,993	152	230·2	4262	3	1420·7	—	—	—	39,255	155	253·3
Not infected 1907	117,722	257	458·1	56,246	42	1339·2	13281	4	3320·3	187,249	303	618·0
Total	152,715	409	373·4	60,508	45	1344·6	13281	4	3320·3	226,504	458	494·6
Mozaffarnagar :												
Never infected	91,920	312	294·6	31,450	21	1497·6	3565	1	3565·0	126,935	334	380·0
Not infected 1907	16,913	46	367·7	18,091	10	1809·1	6943	2	3471·5	41,947	58	723·2
Total	108,833	358	304·0	49,541	31	1598·1	10,508	3	3502·7	168,882	392	430·8
Hoshiarpur :												
Never infected	153,858	634	242·7	36,483	24	1520·1	—	—	—	190,341	658	289·3
Not infected 1907	169,292	502	337·2	62,019	46	1348·2	15,554	3	5184·7	246,865	551	448·0
Total	323,150	1136	284·5	98,502	70	1407·2	15,554	3	5184·7	437,206	1209	361·6

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TABLE XXXIV.

Comparison of mortality rates including non-infected villages, 1907.

Villages having less than 1000 population.

District	Population	Number of villages	Number of deaths	Mortality per cent.	Average population of villages
Rohtak	137,080	299	3224	2·35	458·5
Amritsar	357,961	782	10,560	2·95	457·8
Mozaffarnagar	288,964	699	13,212	4·57	413·4
Hoshiarpur	625,822	1871	9900	1·58	334·5
Villages having population of 1000—3000.					
Rohtak	251,662	152	13,487	5·36	1655·7
Amritsar	378,657	246	10,370	2·74	1539·3
Mozaffarnagar	356,528	225	19,906	5·58	1584·6
Hoshiarpur	326,359	214	5095	1·56	1525·0

TABLE XXXV.

GUJRAT 1907.

	Population	Deaths	Number of villages	D.R.	Ratio	Average population
Villages infected in 2 months:						
Up to 1000	81,009	8476	224	10·46	2·24	361·6
1000—3000.	31,925	1495	24	4·68	—	1330·2
Villages infected in 3 months:						
Up to 1000	107,763	12,424	232	11·53	1·16	464·5
1000—3000	72,732	7238	51	9·95	—	1426·1
Villages infected in 4 months:						
Up to 1000	49,097	6316	93	12·86	1·17	427·9
1000—3000	43,800	4811	27	10·98	—	1622·2

HOSHIARPUR 1907.

Villages infected 2 months:						
Up to 1000	64,553	2158	149	3·34	1·87	433·2
1000—3000	39,269	704	26	1·79	—	1510·3
Villages infected 3 months:						
Up to 1000	59,744	3070	132	5·14	2·32	352·6
1000—3000	46,229	1019	29	2·22	—	1594·1
Villages infected 4 months:						
Up to 1000	27,897	1642	58	5·89	1·86	481·0
1000—3000	42,447	1341	28	3·16	—	1516·0

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TABLE XXXVI.

AMRITSAR 1907.

	Population	Deaths	Number of villages	D.R.	Ratio	Average population
Villages infected during 2 months :						
Up to 1000	78,136	2998	156	3·84	2·48	500·9
1000—3000	62,717	971	45	1·55	—	1393·7
Villages infected during 3 months :						
Up to 1000	65,628	3735	113	5·69	1·64	580·8
1000—3000	115,058	3980	71	3·46	—	1620·5
Villages infected during 4 months :						
Up to 1000	26,655	2478	42	9·30	2·27	634·6
1000—3000	61,601	2527	38	4·10	—	1621·1

TABLE XXXVII.

MOZAFFARNAGAR 1907.

	Population	Deaths	Number of villages	D.R.	Ratio	Average population
Villages infected 2 months :						
Up to 1000	60,531	3956	116	6·54	1·53	521·8
1000—3000	58,216	2486	39	4·27	—	1492·7
Villages infected 3 months :						
Up to 1000	48,425	5527	82	11·41	1·58	590·5
1000—3000	97,987	7069	65	7·21	—	1507·5
Villages infected 4 months :						
Up to 1000	15,923	1830	28	11·49	1·31	568·7
1000—3000	66,720	5840	39	8·75	—	1710·8

TABLE XXXVIII.

ROHTAK 1907.

	Population	Deaths	Number of villages	D.R.	Ratio	Average population
Villages infected 2 months :						
Up to 1000	21,834	1212	34	5·55	1·67	642·2
1000—3000	41,819	1394	25	3·33	—	1672·8
Villages infected 3 months :						
Up to 1000	16,781	1228	28	7·32	·83	599·3
1000—3000	79,156	6984	48	8·82	—	1649·1
Villages infected 4 months :						
Up to 1000	4279	483	6	11·29	1·35	713·2
1000—3000	45,436	3800	23	8·36	—	1975·5

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TABLE XXXIX.

General comparison of climatic conditions in the different districts, five months January—May (inclusive).

Year	Amritsar			Rohtak			Gujrat			Hoshiarpur			Mozaffarnagar		
	Mean mortality per cent. Villages less than 3000 population	Average rainfall	Average number of wet days	Mean mortality per cent. Villages less than 3000 population	Average rainfall	Average number of wet days	Mean mortality per cent. Villages less than 3000 population	Average rainfall	Average number of wet days	Mean mortality per cent. Villages less than 3000 population	Average rainfall	Average number of wet days	Mean mortality per cent. Villages less than 3000 population	Average rainfall	Average number of wet days
1902	2·08	1·511	4·125	—	1·342	2·20	1·56	2·42	7·67	3·18	2·77	7·80	·14	·648	1·50
1903	5·03	4·583	6·625	—	·528	2·00	3·04	4·55	12·33	No data	13·144	11·00	2·24	1·095	3·17
1904	3·83	6·430	13·50	2·52	2·222	5·80	11·05	9·47	15·33	4·87	7·036	15·00	2·47	4·300	9·67
1905	4·59	4·429	11·875	6·11	3·10	7·00	4·87	6·96	13·33	3·25	8·37	16·40	3·11	3·720	8·67
1906	1·93	6·264	12·875	2·79	3·022	4·60	3·00	8·643	14·00	1·99	6·364	14·40	2·20	5·203	9·50
1907	4·02	7·429	17·00	6·85	4·144	8·00	9·81	14·803	22·00	2·78	15·022	22·40	6·86	6·870	12·67

Comparison of mean daily temperature of Lahore with plague mortality in Amritsar district.

Year	January			February			March			April			May		
	Mean mortality of villages where epidemic commenced in Jan.	Mean daily temperature	Mean daily range	Mean mortality of villages where epidemic commenced in Feb.	Mean daily temperature	Mean daily range	Mean mortality of villages where epidemic commenced in Mar.	Mean daily temperature	Mean daily range	Mean mortality of villages where epidemic commenced in April	Mean daily temperature	Mean daily range	Mean mortality of villages where epidemic commenced in May	Mean daily temperature	Mean daily range
1903	5·26	55·1	27·7	6·00	59·9	27·5	5·79	66·8	24·6	5·19	76·9	28·6	2·84	87·2	28·9
1904	3·81	53·1	22·3	6·15	60·4	30·0	5·47	67·1	19·3	3·56	81·1	30·5	1·32	91·0	28·4
1905	4·71	51·6	18·6	5·04	50·1	21·2	4·67	63·6	20·9	4·05	78·0	28·9	2·23	92·7	29·4
1906	2·89	53·1	28·9	2·60	56·8	19·1	2·58	64·9	21·7	2·41	78·5	28·7	·721	92·9	31·2
1907	4·22	58·7	26·3	4·23	56·5	18·8	4·33	64·2	22·8	3·62	77·2	24·1	2·79	87·5	29·3

	1903	1904	1905	1906	1907
Mean daily temperature, five months January—May (inclusive), Lahore	69·18	70·54	67·20	69·24	68·82
,, ,, range ,, ,, ,, ,, ,,	27·46	26·10	23·80	25·92	24·26
Mean percentage mortality for epidemics in Amritsar district	5·03	3·83	4·59	1·93	4·02

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TABLE XL.

Amritsar City. Monthly deaths and meteorological conditions. Feb.—July, 1904—1907.

Month and Year	Number of deaths	Proportion of deaths in the whole 6 months	Rainfall	Number of days upon which rain fell	Mean temperature for the month (Lahore record)
February 1904	0	0	·10	1	60·4°
,, 1905	237	·311	1·05	5	50·1
,, 1906	146	·081	2·90	5	56·8
,, 1907	30	·027	2·50	7	56·5
March 1904	36	·047	4·13	10	67·1°
,, 1905	169	·221	·37	2	63·6
,, 1906	333	·184	1·75	5	64·9
,, 1907	125	·114	1·95	5	64·2
April 1904	359	·471	·06	0	81·1°
,, 1905	127	·166	0	0	78·0
,, 1906	656	·363	·45	2	78·5
,, 1907	417	·382	1·20	3	77·2
May 1904	237	·311	·25	2	91·0°
,, 1905	170	·223	0	0	92·7
,, 1906	581	·321	·20	1	92·9
,, 1907	408	·374	·53	2	87·5
June 1904	109	·143	1·80	2	95·7°
,, 1905	48	·063	·25	1	96·4
,, 1906	83	·046	1·90	3	93·6
,, 1907	111	·102	·60	2	92·0
July 1904	22	·029	·10	1	93·0°
,, 1905	12	·016	3·55	6	92·1
,, 1906	9	·005	3·70	5	93·2
,, 1907	1	·001	1·80	4	93·0

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TABLE XLI. *Hoshiarpur District* 1902.

Hoshiarpur Tehsil.

Month of infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '01	21,987	1049	4·77	18	1221·5
January	29,118	855	2·94	16	1819·9
February	30,473	1053	3·46	33	923·4
March	23,224	409	1·76	26	893·2
April	22,317	363	1·63	24	929·9
May	10,040	140	1·39	19	528·4
June	905	3	·33	2	452·5
July	2219	36	1·62	4	554·7
Total	140,283	3908	2·79	142	987·9

Garshankar Tehsil.

Dec. '01	74,722	3233	4·33	93	803·5
January	51,784	1832	3·54	69	750·5
February	30,480	979	3·21	58	525·5
March	24,853	355	1·43	35	710·1
April	11,959	176	1·47	20	597·9
May	7040	23	·33	10	704·0
June	—	—	—	—	—
July	—	—	—	—	—
Total	200,838	6598	3·29	285	704·7

Dasuyah Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '01	1133	1	·09	1	1133·0
January	861	2	·23	2	430·5
February	1294	23	1·78	1	1294·0
March	6534	138	2·11	7	933·4
April	10,017	341	3·40	4	2504·3
May	4040	9	·22	2	2020·0
June	—	—	—	—	—
July	2543	15	·59	2	1271·5
Total	26,422	529	2·00	19	1390·6

Una Tehsil.

Dec. '01	6550	260	3·97	4	1637·5
January	2537	31	1·22	2	1268·5
February	9569	287	3·00	6	1594·8
March	12,862	55	·43	11	1169·3
April	3625	16	·44	4	906·3
May	1744	4	·23	3	581·3
June	—	—	—	—	—
July	—	—	—	—	—
Total	36,887	653	1·77	30	1229·6

Total Hoshiarpur District 1902.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '01	104,392	4543	4·35	116	899·9
January	84,300	2720	3·23	89	947·2
February	71,816	2342	3·26	98	732·8
March	67,473	957	1·42	79	854·1
April	47,918	896	1·87	52	921·5
May	22,864	176	·77	34	672·5
June	905	3	·33	2	452·5
July	4762	51	1·07	6	793·7
Total	404,430	11,688	2·89	476	849·6

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TABLE XLII. *Hoshiarpur District* 1904.

Hoshiarpur Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '03	—	—	—	—	—
January	21,760	828	3·81	13	1673·8
February	32,861	1652	5·03	20	1643·0
March	30,053	1716	5·71	25	1202·1
April	25,206	1466	5·82	50	504·1
May	60,904	2466	4·05	101	603·0
June	14,147	202	1·43	32	442·1
July	5028	216	4·30	8	628·5
Total	189,959	8546	4·50	249	762·9

Garshankar Tehsil.

Dec. '03	—	—	—	—	—
January	18,190	530	2·91	15	1212·7
February	23,414	1410	6·02	27	867·2
March	45,497	2363	5·19	64	710·9
April	62,050	3041	4·90	91	681·9
May	31,132	986	3·17	65	479·0
June	11,645	156	1·34	20	582·3
July	495	2	·40	1	495·0
Total	192,423	8488	4·41	283	679·9

Dasuyah Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '03	—	—	—	—	—
January	20,963	1097	5·23	31	676·2
February	38,483	2722	7·07	43	895·0
March	23,612	1317	5·58	43	549·1
April	42,048	2474	5·88	99	424·7
May	26,265	971	3·70	64	410·4
June	4384	88	2·01	10	438·4
July	414	2	·48	2	207·0
Total	156,169	8671	5·55	292	534·8

Una Tehsil.

Dec. '03	—	—	—	—	—
January	1987	51	2·57	2	993·5
February	1433	21	1·47	2	716·5
March	11,120	49	·44	4	2780·0
April	7449	66	·89	9	827·7
May	9299	172	1·85	13	715·3
June	—	—	—	—	—
July	2281	3	·13	2	1140·5
Total	33,569	362	1·08	32	1049·0

Total Hoshiarpur District 1904.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '03	—	—	—	—	—
January	62,900	2506	3·98	61	1031·1
February	96,191	5805	6·04	92	1045·6
March	110,282	5445	4·94	136	810·9
April	136,753	7047	5·15	249	549·1
May	127,600	4595	3·60	243	525·1
June	30,176	446	1·48	62	486·7
July	8218	223	2·71	13	632·2
Total	572,120	26,067	4·56	856	668·4

NATIONAL ARCHIVES OF INDIA

TABLE XLIII. *Hoshiarpur District* 1905.

Hoshiarpur Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	46,628	2299	4·93	64	728·6
January	76,700	2532	3·30	71	1080·3
February	24,660	541	2·19	34	725·3
March	22,719	615	2·71	42	540·9
April	22,368	559	2·50	47	475·9
May	15,479	245	1·58	28	552·8
June	8189	129	1·58	19	431·0
July	1566	4	·26	3	522·0
Total	218,309	6924	3·17	308	708·8

Garshankar Tehsil.

Dec. '04	616	12	1·95	3	205·3
January	58,570	2192	3·74	75	780·9
February	31,535	1169	3·71	43	733·4
March	35,327	1403	3·97	62	569·8
April	50,748	1394	2·75	76	667·7
May	16,969	356	2·10	32	530·3
June	7558	102	1·35	20	377·9
July	1356	13	·96	3	452·0
Total	202,679	6641	3·28	314	645·5

Dasuyah Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	12,742	611	4·80	13	980·2
January	42,372	1333	3·15	65	651·9
February	18,550	484	2·61	24	772·9
March	15,957	443	2·78	24	664·9
April	16,477	481	2·92	42	392·3
May	15,399	360	2·34	33	466·6
June	13,595	204	1·50	28	485·5
July	1781	24	1·35	7	254·4
Total	136,873	3940	2·88	236	580·0

Una Tehsil.

Dec. '04	14,892	593	3·98	21	709·1
January	17,128	439	2·56	19	901·5
February	14,668	228	1·55	19	772·0
March	9747	135	1·39	18	541·5
April	12,115	250	2·06	29	417·8
May	3161	47	1·49	9	351·2
June	1185	1	·08	1	1185·0
July	2841	48	1·69	3	947·0
Total	75,737	1741	2·30	119	636·4

Total Hoshiarpur District 1905.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	74,878	3515	4·69	101	741·4
January	194,770	6496	3·34	230	846·8
February	89,413	2422	2·71	120	745·1
March	83,750	2596	3·10	146	573·6
April	101,708	2684	2·64	194	524·6
May	51,008	1008	1·98	102	500·1
June	30,527	436	1·43	68	448·9
July	7544	89	1·18	16	471·5
Total	633,598	19,246	3·04	977	648·5

NATIONAL ARCHIVES OF INDIA

TABLE XLIV. *Hoshiarpur District* 1907.

Hoshiarpur Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	21,134	639	3·02	25	845·4
January	46,257	1233	2·67	35	1321·6
February	24,215	894	3·69	43	563·1
March	44,635	863	1·93	67	666·2
April	37,029	527	1·42	55	673·3
May	16,155	172	1·06	33	489·5
June	12,469	82	·66	10	1246·9
July	619	2	·32	2	309·5
Total	202,513	4412	2·18	270	750·0

Garshankar Tehsil.

Dec. '06	26,304	815	3·10	31	848·5
January	35,858	1096	3·07	43	833·9
February	42,704	1221	2·86	53	805·7
March	46,321	1382	2·98	80	579·0
April	32,102	661	2·06	63	509·6
May	7744	182	2·35	16	484·0
June	1639	3	·18	2	819·5
July	497	11	2·21	1	497·0
Total	193,169	5371	2·78	289	668·4

Dasuyah Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	23,510	875	3·72	40	587·8
January	36,717	976	2·66	42	874·2
February	18,093	736	4·07	37	489·0
March	25,716	1142	4·44	56	459·2
April	27,765	750	2·70	50	555·3
May	4963	105	2·12	18	275·7
June	2094	11	·53	6	349·0
July	1213	1	·08	1	1213·0
Total	140,071	4596	3·28	250	560·3

Una Tehsil.

Dec. '06	2443	146	5·98	3	814·3
January	976	41	4·20	3	325·3
February	11,972	218	1·82	6	1995·3
March	17,252	550	3·19	21	821·5
April	16,491	310	1·88	24	687·1
May	10,829	110	1·02	23	470·8
June	2261	12	·53	2	1330·5
July	419	1	·24	1	419·0
Total	62,643	1388	2·22	83	754·7

Total Hoshiarpur District 1907.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	73,391	2475	3·37	99	741·3
January	119,808	3346	2·79	123	974·0
February	96,984	3069	3·16	139	697·7
March	133,924	3937	2·94	224	597·9
April	113,387	2248	1·98	192	590·6
May	39,691	569	1·43	90	441·0
June	18,463	108	·58	20	923·2
July	2748	15	·55	5	549·6
Total	598,396	15,767	2·63	892	670·8

NATIONAL ARCHIVES OF INDIA

TABLE XLV. *Amritsar* 1903.

AMRITSAR TEHSIL.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '02	31,758	1491	4·69	25	1270·3
January	39,150	1847	4·72	40	978·8
February	27,168	1312	4·83	21	1293·7
March	45,033	2440	5·42	35	1286·7
April	8055	522	6·48	8	1006·9
May	45,835	1653	3·61	41	1117·9
June	4190	90	2·15	6	698·3
Total	201,189	9355	4·65	176	1143·1

AJNALA TEHSIL.

Dec. '02	3367	137	4·07	4	841·8
January	4228	194	4·59	5	704·7
February	17,987	936	5·20	15	1199·1
March	22,362	1161	5·19	27	828·2
April	42,923	1705	3·97	39	1100·6
May	17,307	455	2·63	24	721·1
June	5246	104	1·98	8	655·8
Total	113,420	4692	4·14	122	929·7

TARN-TARN TEHSIL.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '02	34,237	2696	7·87	23	1488·6
January	28,512	1741	6·11	26	1096·6
February	39,403	2823	7·16	36	1094·5
March	37,244	2368	6·36	33	1128·6
April	38,745	2430	6·27	39	993·5
May	26,066	425	1·63	21	1241·2
June	—	—	—	—	—
Total	204,207	12,483	6·11	178	1147·2

TOTAL AMRITSAR DISTRICT 1903.

Dec. '02	69,362	4324	6·23	52	1333·9
January	71,890	3782	5·26	71	1012·5
February	84,558	5071	6·00	72	1174·4
March	104,639	5969	5·79	95	1101·5
April	89,723	4657	5·19	86	1043·3
May	89,208	2533	2·84	86	1037·3
June	9436	194	2·06	14	674·0
Total	518,816	26,530	5·11	476	1089·9

NATIONAL ARCHIVES OF INDIA

TABLE XLVI. *Amritsar* 1904.

Amritsar Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '03	1700	31	1·82	1	1700·0
January	26,560	1053	3·96	13	2043·1
February	17,224	1115	6·47	12	1435·3
March	47,491	2897	6·10	40	1187·3
April	84,713	3080	3·64	93	910·9
May	37,497	547	1·46	37	1013·4
June	1808	9	·50	3	602·7
Total	216,993	8732	4·02	199	1090·4

Ajnala Tehsil.

Dec. '03	2179	25	1·15	1	2179·0
January	2558	4	·16	1	2558·0
February	8469	408	4·82	4	2117·3
March	10,284	392	3·81	10	1028·4
April	36,239	902	2·49	34	1065·9
May	15,851	252	1·59	19	834·3
June	—	—	—	—	—
Total	75,580	1983	2·62	69	1095·4

Tarn-Tarn Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '03	—	—	—	—	—
January	15,902	686	4·31	6	2650·3
February	8514	580	6·81	7	1216·3
March	31,521	1597	5·07	20	1576·1
April	102,974	3998	3·88	80	1287·2
May	38,789	418	1·08	44	881·6
June	71	1	1·41	1	71·0
Total	197,771	7280	3·68	158	1251·7

Total Amritsar District 1904.

Dec. '03	3879	56	1·44	2	1939·5
January	45,020	1743	3·87	20	2250·0
February	34,207	2103	6·15	23	1487·3
March	89,296	4886	5·47	70	1275·7
April	223,926	7980	3·56	207	1081·8
May	92,137	1217	1·32	100	921·4
June	1879	10	·53	4	469·8
Total	490,344	17,995	3·67	426	1151·0

NATIONAL ARCHIVES OF INDIA

TABLE XLVII. *Amritsar* 1905.

Amritsar Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	49,893	2524	5·06	38	1313·0
January	75,278	3489	4·63	71	1060·3
February	46,119	2090	4·53	47	981·3
March	52,869	2161	4·09	55	961·3
April	35,318	1055	2·99	36	981·1
May	11,658	211	1·81	14	832·7
June	—	—	—	—	—
Total	271,135	11,530	4·25	261	1038·8

Ajnala Tehsil.

Dec. '04	12,531	319	2·55	9	1392·3
January	10,896	351	3·22	10	1089·6
February	5658	316	5·59	8	707·3
March	23,362	738	3·16	18	1297·9
April	28,596	1474	5·15	35	817·0
May	39,715	925	2·33	49	810·5
June	2855	38	1·33	6	475·8
Total	123,613	4161	3·37	135	915·7

Tarn-Tarn Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	47,944	2994	6·24	28	1712·4
January	34,212	1857	5·43	37	924·7
February	50,381	2747	5·45	40	1259·5
March	64,179	3769	5·87	56	1146·1
April	55,319	2132	3·85	55	1005·8
May	26,025	584	2·24	31	839·5
June	—	—	—	—	—
Total	278,060	14,083	5·06	247	1125·7

Total Amritsar District 1905.

Dec. '04	110,368	5837	5·29	75	1471·5
January	120,386	5697	4·73	118	1020·2
February	102,158	5153	5·04	95	1075·3
March	140,410	6668	4·75	129	1088·4
April	119,233	4661	3·91	126	946·3
May	77,398	1720	2·22	94	823·4
June	2855	38	1·33	6	475·8
Total	672,808	29,774	4·43	643	1046·4

NATIONAL ARCHIVES OF INDIA

TABLE XLVIII. *Amritsar* 1906.

Amritsar Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '05	2463	68	2·76	2	1231·5
January	8710	211	2·42	7	1244·3
February	12,299	353	2·87	7	1757·0
March	40,737	1335	3·28	25	1629·5
April	66,272	1809	2·73	72	920·4
May	51,000	407	·80	53	962·3
June	11,359	69	·61	9	1262·1
Total	192,840	4252	2·20	175	1101·9

Ajnala Tehsil.

Dec. '05	—	—	—	—	—
January	—	—	—	—	—
February	1455	37	2·54	2	727·5
March	6542	128	1·96	2	3271·0
April	2100	86	4·10	3	700·0
May	11,554	128	1·11	12	962·8
June	3914	3	·08	2	1957·0
Total	25,565	382	1·49	21	1217·4

Tarn-Tarn Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '05	—	—	—	—	—
January	9987	330	3·30	5	1997·4
February	2208	25	1·13	1	2208·0
March	17,674	213	1·21	11	1606·7
April	28,637	449	1·57	22	1301·7
May	33,609	158	·47	27	1244·8
June	6694	6	·09	5	1338·8
Total	98,809	1181	1·20	71	1391·7

Total Amritsar District 1906.

Dec. '05	2463	68	2·76	2	1231·5
January	18,697	541	2·89	12	1558·1
February	15,962	415	2·60	10	1596·2
March	64,953	1676	2·58	38	1709·3
April	97,009	2344	2·42	97	1000·1
May	96,163	693	·72	92	1045·3
June	21,967	78	·36	16	1372·9
Total	317,214	5815	1·83	267	1188·1

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TABLE XLIX. *Amritsar* 1907.

Amritsar Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	22,742	1222	5·37	19	1196·9
January	25,491	992	3·89	19	1341·6
February	24,633	884	3·59	25	985·3
March	78,868	2848	3·61	70	1126·7
April	59,139	2141	3·62	60	985·7
May	23,476	392	1·67	30	782·5
June	5976	73	1·22	8	747·0
Total	240,325	8552	3·56	231	1040·4

Ajnala Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	13,210	766	5·80	9	1467·8
January	6092	403	6·62	9	676·9
February	16,520	932	5·64	13	1270·8
March	26,173	1830	6·99	25	1046·9
April	51,679	3091	5·98	62	833·5
May	37,506	1550	4·13	49	765·4
June	5041	120	2·38	10	504·1
Total	156,221	8692	5·56	177	882·6

Tarn-Tarn Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	5531	234	4·23	4	1382·8
January	5800	181	3·12	4	1450·0
February	19,570	752	3·84	12	1630·8
March	44,626	1795	4·02	30	1487·5
April	95,592	2232	2·33	73	1309·5
May	42,136	930	2·21	49	859·9
June	5074	57	1·12	7	724·9
Total	218,329	6181	2·83	179	1219·7

Total Amritsar District 1907.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	41,483	2222	5·36	32	1296·3
January	37,383	1576	4·22	32	1168·2
February	60,723	2568	4·23	50	1214·5
March	149,667	6473	4·33	125	1197·3
April	206,410	7464	3·62	195	1058·5
May	103,118	2872	2·79	128	805·6
June	16,091	250	1·55	25	643·6
Total	614,875	23,425	3·81	587	1047·5

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TABLE L. *Rohtak* 1905.

Rohtak Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	24,244	815	3·36	6	4040·7
January	61,097	3904	6·39	19	3215·6
February	11,293	868	7·69	10	1129·3
March	22,766	1940	8·52	14	1626·1
April	27,646	801	2·90	22	1256·6
May	1886	36	1·91	2	943·0
June	—	—	—	—	—
Total	148,932	8364	5·62	73	2040·2

Jhajar Tehsil.

Dec. '04	12,657	599	4·73	9	1406·3
January	6631	679	10·24	9	736·8
February	4949	282	5·70	8	618·6
March	17,165	889	5·18	17	1009·7
April	16,481	648	3·93	25	659·2
May	6557	81	1·24	4	1639·3
June	—	—	—	—	—
Total	64,440	3178	4·93	72	895·0

Sampla Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	15,999	889	5·56	6	2666·5
January	21,989	1668	7·59	10	2198·9
February	38,694	2476	6·40	21	1842·6
March	21,022	1916	9·11	17	1236·6
April	24,921	1225	4·92	19	1311·6
May	5428	50	·92	5	1085·6
June	1095	8	·73	1	1095·0
Total	129,148	8232	6·37	79	1634·8

Gohanah Tehsil.

Dec. '04	10,720	1110	10·35	4	2680·0
January	8262	1136	13·75	3	1301·3
February	12,618	962	7·62	4	3154·5
March	21,843	2335	10·69	9	2427·0
April	46,779	2089	4·47	30	1559·3
May	16,753	234	1·40	8	2094·1
June	—	—	—	—	—
Total	116,975	7866	6·72	58	2016·8

Total Rohtak District 1905.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	63,620	3413	5·36	25	2544·8
January	97,979	7387	7·54	41	2389·7
February	67,554	4588	6·79	43	1571·0
March	82,796	7080	8·55	57	1452·6
April	115,827	4763	4·11	96	1206·5
May	30,624	401	1·31	19	1611·8
June	1095	8	·73	1	1095·0
Total	459,495	27,640	6·02	282	1629·4

NATIONAL ARCHIVES OF INDIA

TABLE LI. *Rohtak* 1907.

Rohtak Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	5126	234	4·56	1	5126·0
January	14,724	1710	11·61	3	4908·0
February	14,113	1183	8·38	5	2822·6
March	67,038	3282	4·90	23	2914·7
April	30,417	747	2·46	21	1448·4
May	10,330	103	1·00	7	1475·7
June	612	1	·16	1	612·0
Total	142,360	7260	5·10	61	2333·8

Jhajar Tehsil.

Dec. '06	—	—	—	—	—
January	—	—	—	—	—
February	839	108	12·87	1	839·0
March	2735	109	3·99	3	911·7
April	10,776	306	2·84	14	769·7
May	8500	68	·80	7	1214·3
June	1063	6	·56	2	531·5
Total	23913	597	2·50	27	885·7

Sampla Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	6371	340	5·34	2	3185·5
January	8548	662	7·74	4	2137·0
February	15,170	934	6·16	7	2167·1
March	64,098	6917	10·79	40	1602·5
April	27,130	2344	8·64	20	1356·5
May	12,284	205	1·67	12	1023·7
June	1685	25	1·48	2	842·5
Total	135,286	11,427	8·45	87	1555·0

Gohanah Tehsil.

Dec. '06	26,632	3029	11·37	8	3329·0
January	6236	769	12·33	4	1559·0
February	26,956	2175	8·07	13	2073·5
March	36,014	3491	9·69	19	1895·5
April	28,028	1823	6·50	18	1557·1
May	11,786	267	2·27	9	1309·6
June	930	11	1·18	1	930·0
Total	136,582	11,565	8·47	72	1897·0

Total Rohtak District 1907.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	38,129	3603	9·45	11	3466·3
January	29,508	3141	10·64	11	2682·5
February	57,078	4400	7·71	26	2195·3
March	169,885	13,799	8·12	85	1998·6
April	96,351	5220	5·42	73	1319·9
May	42,900	643	1·50	35	1225·7
June	4290	43	1·00	6	715·0
Total	438,141	30,849	7·04	247	1773·9

NATIONAL ARCHIVES OF INDIA

TABLE LII. *Mozaffarnagar* 1904.

Mozaffarnagar Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '03	1061	6	·57	1	1061·0
January	—	—	—	—	—
February	21,602	623	2·88	7	3086·0
March	3453	18	·52	2	1726·5
April	15,292	289	1·89	14	1092·3
May	3418	7	·20	3	1139·3
June	1162	1	·09	1	1162·0
July	999	4	·40	2	499·5
Total	46,987	948	2·02	30	1566·2

Budhana Tehsil.

Dec. '03	—	—	—	—	—
January	—	—	—	—	—
February	3863	263	6·81	1	3863·0
March	12,544	113	·90	3	4181·3
April	1829	14	·77	2	914·5
May	—	—	—	—	—
June	—	—	—	—	—
July	—	—	—	—	—
Total	18,236	390	2·14	6	3039·3

Kairana Tehsil.

April	5094	3	·06	1	5094·0

Jansath Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '03	18,358	645	3·51	5	3671·6
January	18,315	616	3·36	7	2616·4
February	13,116	445	3·39	12	1093·0
March	42,236	1436	3·40	25	1689·4
April	35,505	516	1·45	29	1224·3
May	7536	131	1·74	6	1256·0
June	5502	19	·35	4	1375·5
July	—	—	—	—	—
Total	140,568	3808	2·71	88	1597·4

Total Mozaffarnagar District 1904.

Dec. '03	19,419	651	3·35	6	3236·5
January	18,315	616	3·36	7	2616·4
February	38,581	1331	3·45	20	1929·0
March	58,233	1567	2·69	30	1941·1
April	57,720	822	1·44	46	1254·8
May	10,954	138	1·26	9	1217·1
June	6664	20	·30	5	1332·8
July	999	4	·40	2	499·5
Total	210,885	5149	2·44	125	1687·1

NATIONAL ARCHIVES OF INDIA

TABLE LIII. *Mozaffarnagar* 1905.

Mozaffarnagar Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	42,302	1743	4·12	16	2643·9
January	27,246	1100	4·04	14	1946·1
February	25,801	879	3·41	15	1720·1
March	46,657	976	2·09	37	1261·0
April	19,560	423	2·16	18	1086·7
May	16,215	329	2·03	16	1013·4
June	1720	4	·23	3	573·3
Total	179,501	5454	2·04	119	1508·4

Budhana Tehsil.

Dec. '04	12,128	126	1·04	7	1732·6
January	12,720	586	4·61	5	2544·0
February	6287	349	5·55	4	1571·8
March	26,083	1372	5·26	13	2006·4
April	21,697	1016	4·68	13	1669·0
May	5320	78	1·47	6	886·7
June	—	—	—	—	—
Total	84,235	3527	4·19	48	1754·9

Jansath Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	83,558	3106	3·72	39	2142·5
January	36,196	1051	2·90	27	1340·6
February	6448	134	2·08	9	716·4
March	45,848	1377	3·00	28	1637·4
April	18,302	496	2·71	16	1143·9
May	9784	119	1·22	9	1087·1
June	1349	3	·22	1	1349·0
Total	201,485	6286	3·12	129	1561·9

Kairana Tehsil.

Dec. '04	—	—	—	—	—
January	6376	275	4·31	2	3188·0
February	1245	40	3·21	1	1245·0
March	7966	183	2·30	4	1991·5
April	7555	130	1·72	7	1079·3
May	3024	20	·66	3	1008·0
June	—	—	—	—	—
Total	26,166	648	2·48	17	1539·2

Total Mozaffarnagar District 1905.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	137,988	4975	3·61	62	2262·1
January	82,538	3012	3·65	48	1719·5
February	39,781	1402	3·52	29	1371·8
March	126,554	3908	3·09	82	1543·3
April	67,114	2065	3·08	54	1242·9
May	34,343	546	1·59	34	1010·1
June	3069	7	·23	4	767·3
Total	491,387	15,915	3·24	313	1569·9

NATIONAL ARCHIVES OF INDIA

TABLE LIV. *Mozaffarnagar* 1906.

Mozaffarnagar Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '05	3565	140	3·93	1	3565·0
January	3428	214	6·24	2	1714·0
February	1448	2	·14	2	724·0
March	3540	88	2·49	3	1180·0
April	5593	52	·93	4	1398·3
May	557	1	·18	1	557·0
June	—	—	—	—	—
Total	18,131	497	2·74	13	1394·7

Budhana Tehsil.

Dec. '05	9651	177	1·83	3	3217·0
January	—	—	—	—	—
February	6973	424	6·08	3	2324·3
March	10,226	266	2·60	8	1278·3
April	12,154	65	·53	7	1736·3
May	6789	36	·53	3	2263·0
June	—	—	—	—	—
Total	45,793	968	2·11	24	1908·0

Jansath Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '05	9597	144	1·50	3	3199·0
January	—	—	—	—	—
February	8888	588	6·62	3	2962·7
March	14,826	471	3·18	7	2118·0
April	8085	136	1·68	6	1347·5
May	5855	46	·79	3	1951·7
June	2699	1	·04	1	2699·0
Total	49,950	1386	2·77	23	2171·7

Kairana Tehsil.

Dec. '05	2016	12	·60	2	1008·0
January	—	—	—	—	—
February	—	—	—	—	—
March	—	—	—	—	—
April	1514	45	2·97	2	757·0
May	2694	2	·07	1	2694·0
June	—	—	—	—	—
Total	6224	59	·95	5	1244·8

Total Mozaffarnagar District 1906.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '05	24,829	473	1·91	9	2758·8
January	3428	214	6·24	2	1714·0
February	17,309	1014	5·86	8	2163·6
March	28,592	825	2·89	18	1588·4
April	27,346	298	1·09	19	1439·3
May	15,895	85	·53	8	1986·9
June	2699	1	·04	1	2699·0
Total	120,098	2910	2·42	65	1847·7

NATIONAL ARCHIVES OF INDIA

TABLE LV. *Mozaffarnagar* 1907.

Mozaffarnagar Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	24,248	920	3·79	11	2204·4
January	24,516	2375	9·69	11	2228·7
February	46,588	3878	8·32	27	725·5
March	54,417	4865	8·94	52	1046·5
April	57,489	3396	5·91	71	809·7
May	19,160	380	1·98	29	660·7
June	2144	16	·75	3	714·7
Total	228,562	15,830	6·93	204	1120·4

Budhana Tehsil.

Dec. '06	1791	116	6·48	2	895·5
January	18,885	1509	7·99	5	3777·0
February	24,305	1819	7·48	12	2025·4
March	16,611	1751	10·54	11	1510·1
April	32,320	1512	4·68	26	1243·1
May	29,727	628	2·11	24	1238·6
June	6317	84	1·33	4	1579·3
Total	129,956	7419	5·71	84	1547·1

Jansath Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	55,280	2663	4·82	26	2126·2
January	39,613	3319	8·38	32	1237·9
February	59,294	4534	7·65	40	1482·4
March	61,561	4820	7·83	60	1026·0
April	35,311	2511	7·11	39	905·4
May	18,908	409	2·16	25	756·3
June	—	—	—	—	—
Total	269,967	18,256	6·76	222	1216·1

Kairana Tehsil.

Dec. '06	2261	154	6·81	2	1130·5
January	4120	295	7·16	2	2060·0
February	3047	291	9·55	2	1523·5
March	12,616	1590	12·60	10	1261·6
April	35,541	2246	6·32	38	935·3
May	12,363	535	4·33	21	588·7
June	561	22	3·92	3	187·0
Total	70,509	5133	7·28	78	903·4

Total Mozaffarnagar District 1907.

Month of 1st infection	Population	No of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	83,580	3746	4·61	41	2038·5
January	87,134	7498	8·61	50	1742·7
February	133,234	10,522	7·90	81	1644·9
March	145,205	13,026	8·97	133	1091·8
April	160,661	9665	6·02	174	923·3
May	80,158	1952	2·44	99	809·7
June	9022	122	1·35	10	902·2
Total	698,994	46,531	6·67	588	1188·8

NATIONAL ARCHIVES OF INDIA

TABLE LVI. *Gujrat* 1904.

Gujrat Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '03	—	—	—	—	—
January	—	—	—	—	—
February	667	39	5·85	2	333·5
March	16,813	710	4·22	12	1401·1
April	27,403	812	2·96	33	830·4
May	13,380	205	1·53	20	669·0
June	769	17	2·21	3	256·3
July	—	—	—	—	—
Total	59,032	1783	3·02	70	843·3

Phalia Tehsil.

Dec. '03	8199	736	8·98	9	911·0
January	3808	1051	27·60	4	952·0
February	14,197	1723	12·14	10	1419·7
March	37,810	8338	22·05	51	741·4
April	57,492	8410	14·63	97	592·7
May	11,006	391	3·55	16	687·9
June	5704	97	1·70	6	950·7
July	—	—	—	—	—
Total	138,216	20,746	15·01	193	716·1

Kharian Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No of. villages	Average popn. of villages
Dec. '03	5454	176	3·23	1	5454·0
January	—	—	—	—	—
February	1665	28	1·68	2	832·5
March	3509	137	3·90	5	701·8
April	13,441	497	3·70	13	1033·9
May	6182	246	3·98	10	618·2
June	5854	30	·51	7	836·3
July	620	1	·16	1	620·0
Total	36,725	1115	3·04	39	941·7

Total Gujrat District 1904.

Dec. '03	13,653	912	6·68	10	1365·3
January	3808	1051	27·60	4	952·0
February	16,529	1790	10·83	14	1180·6
March	58,132	9185	15·80	68	854·9
April	98,336	9719	9·88	143	687·7
May	30,568	842	2·76	46	664·5
June	12,327	144	1·17	16	770·4
July	620	1	·16	1	620·0
Total	233,973	23,644	10·11	302	774·8

NATIONAL ARCHIVES OF INDIA

TABLE LVII. *Gujrat* 1905.

Gujrat Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	6923	668	9·65	10	692·3
January	5645	432	7·65	12	470·4
February	6377	537	8·42	9	708·6
March	27,821	1812	6·51	27	1030·4
April	28,671	2109	7·36	44	651·6
May	26,875	494	1·84	34	790·4
June	4062	21	·52	9	451·3
July	—	—	—	—	—
Total	106,374	6073	5·71	145	733·6

Phalia Tehsil.

Dec. '04	4436	153	3·45	4	1109·0
January	3051	159	5·21	3	1017·0
February	583	87	14·92	2	291·5
March	19,852	598	3·01	23	863·1
April	33,056	1216	3·68	38	869·9
May	12,654	207	1·64	19	666·0
June	965	7	·73	2	482·5
July	—	—	—	—	—
Total	74,597	2427	3·25	91	819·7

Kharian Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '04	—	—	—	—	—
January	302	3	·99	1	302·0
February	1725	5	·29	2	862·5
March	807	265	32·84	2	403·5
April	8959	372	4·15	11	814·5
May	7328	132	1·80	8	916·0
June	—	—	—	—	—
July	—	—	—	—	—
Total	19,121	777	4·06	24	796·7

Total Gujrat District 1905.

Dec. '04	11,359	821	7·23	14	811·4
January	8998	594	6·60	16	562·4
February	8685	629	7·24	13	668·1
March	48,480	2675	5·52	52	932·3
April	70,686	3697	5·23	93	760·1
May	46,857	833	1·78	61	768·2
June	5027	28	·56	11	457·0
July	—	—	—	—	—
Total	200,092	9277	4·64	260	769·6

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TABLE LVIII. *Gujrat* 1907.

Gujrat Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	7626	61	·80	2	3813·0
January	—	—	—	—	—
February	3569	350	9·81	5	713·8
March	35,037	3022	8·63	45	778·6
April	84,250	6298	7·48	128	658·2
May	48,702	1899	3·90	101	482·2
June	16,208	289	1·78	32	506·5
July	570	21	3·68	3	190·0
Total	195,962	11,940	6·09	316	620·1

Phalia Tehsil.

Dec. '06	20,570	1680	8·17	14	1469·3
January	15,824	1909	12·06	20	791·2
February	34,934	5163	14·78	49	712·9
March	62,139	8576	13·80	95	654·1
April	38,208	4236	11·09	66	578·9
May	2383	160	6·71	10	238·3
June	98	19	19·39	1	98·0
July	—	—	—	—	—
Total	174,156	21,743	12·48	255	682·7

Kharian Tehsil.

Month of 1st infection	Population	No. of deaths	Mortality per cent.	No. of villages	Average popn. of villages
Dec. '06	—	—	—	—	—
January	—	—	—	—	—
February	2333	443	18·99	2	1166·5
March	23,614	3701	15·67	25	944·6
April	68,216	9210	13·50	100	682·2
May	51,912	2925	5·63	113	459·4
June	9708	255	2·63	34	285·5
July	3235	18	·56	6	539·2
Total	159,018	16,552	10·41	280	568·5

Total Gujrat District 1907.

Dec. '06	28,196	1741	6·17	16	1762·3
January	15,824	1909	12·06	20	791·2
February	40,836	5956	14·59	56	729·2
March	120,790	15,299	12·67	165	732·1
April	190,674	19,744	10·36	294	648·6
May	102,997	4984	4·84	224	459·8
June	26,014	563	2·16	67	388·3
July	3805	39	1·03	9	422·8
Total	529,136	50,235	9·49	851	621·8

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TABLE LIX.

Infected villages within selected area, Gujrat 1907.

Month of 1st infection	Population	No. of deaths	No. of villages	Mortality per cent.	Average population of villages
Dec. '06	—	—	—	—	—
January	—	—	—	—	—
February	7394	1642	8	22·21	924·3
March	28,616	4992	27	17·44	1059·9
April	33,276	6503	53	19·54	627·8
May	11,692	1045	26	8·94	449·7
June	1425	89	8	6·25	178·1
Total	82,403	14,271	122	17·32	675·4

Infected villages without selected area, Gujrat 1907.

Dec. '06	28,196	1741	16	6·17	1762·3
January	15,824	1909	20	12·06	791·2
February	33,442	4314	48	12·90	696·7
March	92,174	10,307	138	11·18	667·9
April	157,398	13,241	241	8·41	653·1
May	91,305	3939	198	4·31	461·1
June	24,589	474	59	1·93	416·8
July	3805	39	9	1·02	422·8
Total	446,733	35,964	729	8·05	612·8

TABLE LX.

Infected villages within selected area, Gujrat District 1904.

Month of 1st infection	Population	No. of deaths	No. of villages	Mortality per cent.	Average population of villages
Dec. '03	5454	176	1	3·23	5454·0
January	—	—	—	—	—
February	534	18	1	3·37	534·0
March	689	209	3	30·33	229·7
April	15,146	1215	15	8·02	1009·7
May	3972	205	6	5·16	662·0
June	3383	18	4	·53	845·8
July	—	—	—	—	—
Total	29,178	1841	30	6·31	972·6

Infected villages without selected area, Gujrat District 1904.

Dec. '03	8199	736	9	8·98	911·0
January	3808	1051	4	27·60	952·0
February	15,995	1772	13	11·08	1230·4
March	57,443	8976	65	15·63	883·7
April	83,190	8504	128	10·22	649·9
May	26,596	637	40	2·40	664·9
June	8944	126	12	1·41	745·3
July	620	1	1	·16	620·0
Total	204,795	21,803	272	10·65	752·9

NATIONAL ARCHIVES OF INDIA

TABLE LXI. *Clerical mortality* 1348—9.

(1) Institutions and Average Institutions.

Place	Average No. of Institutions (Annual)	Institutions 1348—9	Ratio
Cornwall & Devon ...	36	382	10·6
Hants.	21	228	10·9
Cambridge	9	97	10·8
Hereford	13	200	15·4
Shropshire	1½	29	19·3
Derby	7	77 (deaths)	11·0
Wilts.	26	176 (in 2 years)	6·8
Norwich (Diocese) ...	35	297 (in 2 years)	8·5
Bath & Wells (Diocese)	77	800	10·3

(2) Total Benefices and Institutions.

			Percentage
Bucks.	180	83	46·1
Worcester	138	67	48·5
Northants.	281	161	57·3
Derby	108	77	71·3
Dorset	211	110	52·1
Notts.	126	65	51·6
Chester	70	30 (1 Archdeaconry)	—
W. R. Yorks. ...	141	96	68·1
E. R. Yorks.	126	65	51·6

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TABLE LXII. *English Records.*

City or village	Estimated population	Population required to give the same rate of mortality as London	Plague deaths
Epidemic of 1603—4.			
London	250,000	250,000	33,347
Chester	Less than 6000 *	12,265	1636
York	Less than 6000 †	26,329	3512
Shrewsbury	Less than 4000 ‡	5000	667
Epidemic of 1625—6.			
London	320,000	320,000	41,313
Plymouth	Less than 10,000 *	15,492	2000
Ashburton	——	2827	365 §
Norwich	23,000 ‖	11,085	1431
Epidemic of 1665—6.			
London	460,000	460,000	68,596
Eyam	350	1737	259
Deptford	——	3500	522
Colchester	Less than 10,000 *	32,302	4817

* "In the reign of Charles the Second no provincial town in the kingdom contained thirty thousand inhabitants and only four provincial towns as many as ten thousand inhabitants." Macaulay, I. 164.

(The four towns were Bristol, Norwich, York, Exeter.)

† Macaulay estimates the population in 1685 at about 10,000.

‡ 7000 inhabitants at the census of 1695.

§ "Probably a fourth of the inhabitants," *Notes and Queries*, 6 Ser. III. 477, cited by Creighton, I. 524.

‖ 28,000—29,000 at the census of 1693.

TABLE LXIII.

The plague experience of the South of France 1720—21.

Population group	No. of towns	Total population	Total deaths	Death rate
90,000	1 (Marseilles)	90,000	39,134	43·5
20,000—90,000	2	46,000	20,694	45·0
5000—	4	24,000	5457	22·7
3000—	7	25,200	4805	19·1
2000—	3	6200	1300	21·0
1000—	4	5050	1242	24·6
500—	10	7224	1904	26·4
0—	7	2175	493	22·7

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TABLE LXIV.

Deaths from plague, September 1906—*September* 1907.

(Grouped in 3-weekly periods.)

	Amritsar	Gujrat
—September 15th	1	—
—October 6th	4	—
—October 27th	5	18
—November 17th	35	97
—December 8th	132	59
—December 29th	346	173
—January 19th	389	201
—February 9th	639	677
—March 2nd	1109	1585
—March 23rd	1794	5766
—April 13th	3244	6417
—May 4th	5591	11,827
—May 25th	6867	15,823
—June 15th	2988	9357
—July 6th	707	2820
—July 27th	76	418
—August 17th	16	7
—September 7th	4	—
Total	23,947	55,245

Amritsar 1905—1906.

(4-weekly periods.)

—September 23rd	...	1
—October 21st	...	0
—November 18th	...	5
—December 16th	...	26
—January 13th	...	30
—February 10th	...	60
—March 10th	...	143
—April 7th	...	437
—May 5th	...	1896
—June 2nd	...	3010
—June 30th	...	431
—July 28th	...	20
—August 25th	...	2
Total		6061

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Appendix IV.

Further Note on the rates of mortality in villages of different sizes.

On p. 81 I attempted to show that the difference in the rate of mortality, observed in most epidemics when one passed from large to small infected villages, could hardly be accounted for by assuming that the chance of infection reaching a colony of rats was independent and that the ratio between the number of human deaths and the number of plague-infected colonies was constant. I will here discuss the results which flow from the assumption that a single colony has a greater chance of being infected if it is situated in a large village than if it is in a small one. In other words I will assume that the chance of infection is not constant from group to group though constant within each group.

Let p_1 be the chance of a colony becoming infected among the colonies situated in villages which contain on the average m_1 colonies a piece. Similarly let p_2 and m_2 be corresponding values for another group of villages. Let $p_1 + q_1 = 1$ and $p_2 + q_2 = 1$.

Then the ratio of the mortalities among infected villages of the two groups is[1]

$$\frac{1-q_1}{1-q_2} \times \frac{1-q_2^{m_2}}{1-q_1^{m_1}} \quad \ldots\ldots\ldots\

number of colonies per village in the group 0—1000 is 1, and $m_1 = 2$ or $= 3$. Then, taking Amritsar 1907,

we have $q_1 = \frac{45}{246} = \cdot183$ and $q_2 = \frac{409}{782} = \cdot523$.

The ratio of mortality rate in infected villages 1000—3000 to mortality rate in infected villages 0—1000 will be ·822 if $m_1 = 3$ and $m_2 = 1$, ·85 if $m_1 = 2$ and $m_2 = 1$. The observed ratio is ·63. The limits within which the ratio might be anticipated to fall are ·817 and 1·71.

In this way, I have deduced the entries in Table LXVII from the data of Table LXV. In two cases, Amritsar 1905 and 1907, the observed ratio is smaller than the least value deduced from the above hypothesis. In the seven other illustrations the real value is between the theoretical limits, but no plausible values for p_1, p_2, m_1 and m_2 are suggested by the results. The general conclusion appears to be that, even as modified here, the hypothesis will not explain the facts.

TABLE LXV.

Comparison of mortality for villages actually infected with that of whole population at risk.

AMRITSAR.

	Population of infected villages	No. of villages	No. of deaths	D.R. %	Population of all villages, in group	No. of villages	D.R. %
Population 0—1000.							
1902	13,151	20	298	2·27	357,961	782	·08
1903	163,074	281	10,286	6·31	357,961	782	2·87
1904	137,851	247	5177	3·76	357,961	782	1·45
1905	228,256	419	12,632	5·53	357,961	782	3·53
1906	77,707	142	2015	2·59	357,961	782	·56
1907	205,246	373	10,560	5·15	357,961	782	2·95
Population 1000—3000.							
1902	49,489	31	1008	2·04	378,657	246	·27
1903	275,965	175	16,163	5·86	378,657	246	4·27
1904	266,025	168	11,357	4·27	378,657	246	3·00
1905	336,362	215	13,554	4·03	378,657	246	3·58
1906	167,467	109	2785	1·66	378,657	246	·74
1907	318,149	201	10,370	3·26	378,657	246	2·74

ROHTAK.

Population 0—1000.							
1904	12,604	23	376	2·98	137,080	299	·27
1905	65,434	120	4281	6·54	137,080	299	3·12
1906	911	2	9	·99	137,080	299	·01
1907	54,446	91	3224	5·92	137,080	299	2·35
Population 1000—3000.							
1904	27,432	17	619	2·26	251,662	152	·25
1905	197,942	119	11,751	5·94	251,662	152	4·67
1906	23,200	13	748	3·22	251,662	152	·30
1907	192,660	112	13,487	7·00	251,662	152	5·36

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TABLE LXVI.

Comparison of mortality for villages actually infected with that of whole population at risk.

MOZAFFARNAGAR.

Population 0—3000.

	Population of infected villages	No. of villages	No. of deaths	D.R. %	Population of all villages, in group	No of villages	D.R. %
1902	2137	1	3	·14	645,492	924	·00
1903	23,435	18	525	2·24	645,492	924	·08
1904	126,228	108	3115	2·47	645,492	924	·48
1905	298,663	273	9285	3·11	645,492	924	1·44
1906	70,485	54	1551	2·20	645,492	924	·24
1907	489,201	541	33,544	6·86	645,492	924	5·20
Population over 3000.							
1902	23,444	1	1	·00	274,710	52	·00
1903	50,509	5	257	·51	274,710	52	·09
1904	131,227	20	2371	1·81	274,710	52	·86
1905	247,026	43	6961	2·82	274,710	52	2·53
1906	103,924	14	1526	1·47	274,710	52	·56
1907	264,202	49	16,365	6·19	274,710	52	5·96

HOSHIARPUR.

Population 0—3000.

1898	11,635	15	352	3·03	952,181	2085	·04
1899	3701	4	9	·24	952,181	2085	·00
1900	9636	11	50	·52	952,181	2085	·01
1901	33,760	43	332	·98	952,181	2085	·03
1902	340,675	464	10,824	3·18	952,181	2085	1·14
1903	No data:	—	—	—	952,181	2085	—
1904	506,168	844	24,642	4·87	952,181	2085	2·59
1905	555,936	940	18,079	3·25	952,181	2085	1·90
1906	176,690	284	3513	1·99	952,181	2085	·37
1907	530,305	879	14,745	2·78	952,181	2085	1·55
Population over 3000.							
1898	5803	1	87	1·50	83,633	16	·10
1899	—	—	—	—	83,633	16	—
1900	—	—	—	—	83,633	16	—
1901	9030	2	112	1·24	83,633	16	·13
1902	63,755	12	864	1·36	83,633	16	1·03
1903	No data:	—	—	—	83,633	16	—
1904	65,952	12	1425	2·16	83,633	16	1·70
1905	63,305	12	1052	1·66	83,633	16	1·26
1906	52,038	9	612	1·18	83,633	16	·73
1907	68,079	13	962	1·41	83,633	16	1·15

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TABLE LXVII.

Ratio of the rate of mortality in infected villages 1000—3000, *to the rate in infected villages* 0—1000.

District	Year	(1) Observed ratio	(2) Smallest theoretical	(3) Largest theoretical	(4) Theoretical for $m_1=3$, $m_2=1$	(5) Theoretical for $m_1=2$, $m_2=1$
Amritsar	1902	·90	·13	4·93	·38	·53
,,	1903	·93	·71	1·98	·73	·78
,,	1904	1·13	·68	2·16	·71	·76
,,	1905	·73	·87	1·63	·88	·89
,,	1906	·64	·44	2·44	·54	·64
,,	1907	·63	·82	1·71	·82	·85
Rohtak	1904	·76	·11	1·45	·37	·53
,,	1905	·91	·78	1·95	·79	·82
,,	1907	1·18	·74	2·42	·75	·79

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XLVI. OBSERVATIONS ON PLAGUE IN EASTERN BENGAL AND ASSAM.

With Map and 5 Charts in Text and Plates II—XIV.

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A. Introduction

The Sanitary Commissioner with the Government of Eastern Bengal and Assam addressed the following letter to the Sanitary Commissioner with the Government of India:

"It has been suggested that an addition to our knowledge of plague might be gained by a study of the conditions under which the people of this Province are living seeing that they have not hitherto suffered from the disease in an indigenous form, and I have the honour to ask whether the members of the Plague Commission could make it convenient to extend their researches in this direction. I may add that the Local Government would be glad to welcome them and to afford them assistance in their work."

In compliance with this invitation the Advisory Committee arranged that the Commission working in India should make an inquiry in the Province of Eastern Bengal and Assam. This inquiry was started in December, 1909, and the results are contained in the following pages.

B. History of Plague in the Province.

Since the commencement of the present outbreak of plague in India in 1896, the province of Eastern Bengal and Assam has suffered only to a very limited extent from the disease. With the exception of a small but definite epidemic of bubonic plague at Dibrugarh in the extreme north-east of the province, and a few doubtful cases occurring in a remote part of Manipur, the few epidemics that have occurred in the province have all been of pneumonic type. Some isolated cases of bubonic plague in addition have also been reported but a history of importation from outside the province could in each case be obtained. No evidence of a rat mortality except at Dibrugarh was ever recorded.

The following is a brief summary of the information obtained from the reports of various medical officers and government officials on the occurrence of plague in the province since the present outbreak of plague began in India in the end of the year 1896.

1. Backergunge, September, 1898. In September, 1898, 11 cases of pneumonic plague occurred at Shidakati and Abbynil, in the Nalchiti rural circle of Backergunge. The infection was said to have been imported from Calcutta; all persons attacked died. This epidemic was

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minutely studied by the Royal Commission and is reported in detail in Vol. v. p. 691 of their report.

2. Pania villages (Dacca district), February–March, 1899. The next outbreak occurred at Pania villages in the Mounshiganj subdivision of Dacca. The epidemic originated in a case which was imported from Calcutta. The patient died in one of the villages on the day following his arrival from Calcutta. This case gave rise by direct contact or communication to 42 cases living in nine homesteads. Of the persons attacked 38 died. The disease was apparently of the pneumonic type. There was no history of rat mortality, and a note was made of the absence of any glandular swellings. No bacteriological examination of the sputum was undertaken.

3. Tippera, March, 1899. Six cases of plague were imported from Calcutta into various places in the Tippera district in March, 1899. Most of them were of the bubonic type and were not associated with cases of local origin.

4. Faridpur, March, 1899. A small outbreak of pneumonic plague occurred in two villages, viz. Bajitpur and Shirakhara in Madaripur subdivision of Faridpur district. There were in all 30 cases and 29 deaths. The source of the infection was not recorded. There were apparently no deaths amongst rats but two cases in Bajitpur were said to be of the bubonic type.

5. Dibrugarh (Lakhimpur district), May, 1903. An outbreak occurred in Dibrugarh in May, 1903. A man, a Dacca Mahomedan, was taken ill on 17th May with high fever and buboes. He died two days later. A large number of rats had been observed to be dying for some days previously and the rats first found were within 50 yards of his house; cultivations of the organisms obtained from the rat revealed a cocco-bacillus similar to plague. There was strong evidence connecting the disease with rats and grain golas (receptacles for storing grain). There were in all 36 cases and 22 deaths. Of these cases 23 were traceable directly, and four indirectly, to grain shops and golas. The epidemic was limited to foreigners, chiefly Marwaris who deal in grain. Most of the dead rats were found in Kayapati where also 20 out of the 36 cases lived. The cases seem to have been of the bubonic type but one case presented pneumonic symptoms.

6. Serajganj (Pabna district), March–April, 1906. A case was imported into Raghurbari village from a jute mill near Calcutta in March, 1906, and gave rise to 27 attacks and 24 deaths; all were of the pneumonic type though one was noted as having enlarged glands of

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the neck. All were apparently infected by direct contact. There was no rat mortality observed.

7. Mymensingh, June–August, 1906. There were two outbreaks in Bajitpur village. Both outbreaks derived their infection from Calcutta and both were of the pneumonic type with a marked absence of buboes. In the first (June, 1906) outbreak five cases occurred and all of these died. In the second there were 50 cases and 45 deaths.

8. English Bazaar (Malda District), January, 1907. Infection was derived from Ballia in the United Provinces. Four persons infected by direct contact were attacked with plague and all died; no mention was made of the type of the disease but symptoms pointed to pneumonia.

9. Goalundo (Faridpur District), April, 1907. One imported case arrived in a steamer from Dighaghat suffering at the time from symptoms of bubonic plague.

10. Tippera, April, 1907. A case of pneumonic plague imported from Calcutta occurred at Kalikacha.

11. Manipur, June, 1907. About eight doubtful cases of bubonic plague with four deaths are said to have occurred near Imphal in Manipur district, a very wild, isolated and hilly country near the Burmese frontier. In the report it was hinted that the infection was probably derived from Burmah.

12. Goalpara, March, 1909. A plague case was imported into Goalpara from Calcutta in March, 1909. There was no mention of the type of the disease but no further cases were reported.

13. Chittagong, January, 1910. A bubonic plague case occurred at Chittagong in January, 1910, imported from the United Provinces.

14. Noakhali, June, 1910. A pneumonic plague outbreak occurred at Noakhali in June, 1910. The infection was imported from Calcutta. Fifty-one cases were reported of whom 45 died. The epidemic lasted one month and spread by direct contact. All the cases were of the pneumonic type of the disease and there was no mortality noted amongst rats.

The history of plague in this province thus shows that while a number of pneumonic cases of plague, imported into the province, gave rise to small epidemics of the disease, only a very few cases of bubonic plague were recorded. We have reason to believe, however, that the preponderance of imported pneumonic cases is only apparent, that in fact the pneumonic cases came to the notice of the authorities mainly because of the epidemics associated with them, while many cases of bubonic plague were imported into the province but were not recorded,

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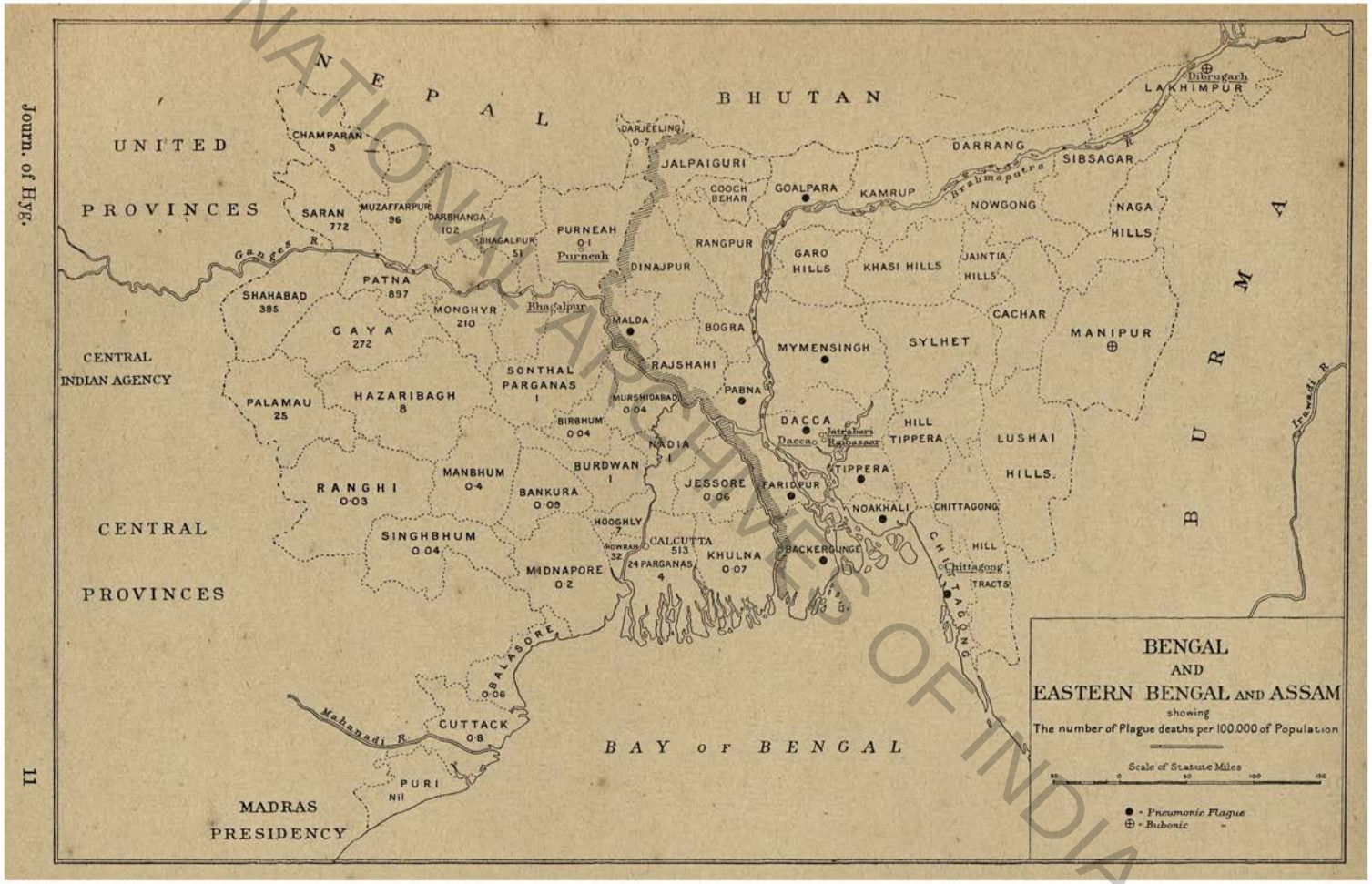
Journ. of Hyg.



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because they were not connected with epidemics. This is the more probable when we remember that only about 3 % of the cases of plague in India are of the pneumonic type, that no very elaborate precautions are taken to register all cases of plague in the province, and that communication with infected areas is freely allowed. We may conclude therefore that plague infection has been frequently brought into the province and that there are definite reasons for the disease not becoming endemic.

C. Methods adopted for studying the Conditions prevailing in the Province.

(1) *Observations made in Dacca.*

Dacca is a town of about 90,000 inhabitants situated in a direct line only 160 miles from Calcutta where epidemics of plague are of annual occurrence. Daily communication is maintained with Calcutta so that the opportunities for the importation of infection are considerable. The town is the largest in the province; the houses are placed more closely together and the population concentrated on a smaller area than in the majority of places in the province. It is reasonable to believe therefore that, provided infection were once implanted in the town, the disease would make more headway among the rat population than in the more scattered villages of the district, and moreover the presence of the disease would readily come to the notice of the authorities more particularly because the supervision of the health of the town is in the hands of a qualified Medical Officer. We may assume then that opportunities for the importation of infection into Dacca have been considerable but that for some reason the disease has never succeeded in gaining a foothold in the town, so that whatever conditions are unfavourable to the prevalence of plague in the province may be presumed to be present to a marked degree in Dacca itself.

An additional reason for the selection of Dacca is to be found in the fact that owing to the size of the town a considerable number of rats could be caught daily without materially interfering with the rats and fleas infesting the place.

(*a*) *Work in the city.* The routine methods of rat and flea infestation carried out by the Commission in various places in India have already been described so frequently that it is scarcely necessary to repeat them here in detail. Observations on the usual lines were

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commenced in the town in December, 1909, and continued daily throughout the course of the year. In order that the observations here might be compared with those made by the Commission in Poona and elsewhere in India, the methods adopted in conducting our inquiry were similar in all essential details to those employed in our previous work. A certain number of traps were set daily, simultaneously in three sections, in the city. The rats caught in the traps were brought in to the laboratory every morning for examination, the traps as usual being enclosed in flea-proof canvas bags.

(*b*) *In the laboratory and office.* The rats contained in the traps were examined in the usual way and all information thus obtained was collected into registers and summarised from time to time. Further, with the object of learning whether the structure of a house has any influence on the number of rats found in it, the houses in the city were classified in groups according to the structure of the wall, the character of the roof, the nature of the floor or plinth, and the number of rats caught in traps set in the houses so classified was noted. A certain number of traps[1] were also baited with different kinds of bait such as coconut, dried fish, plaintain etc., and these were set at random amongst those distributed in the ordinary routine; the results thus obtained were compared with the ordinary bread and oil bait, used throughout our observations.

(2) *Work in the surrounding district.*

(*a*) *Work in the non-plague area of Eastern Bengal and Assam.* A number of towns and villages were selected, as far as possible representative of every type in the province; these were visited and observations were made on the structure of the houses, the habits of the people, the number of rats found in the houses and the flea infestation of the rats, in order to compare the conditions found in them with those found in Dacca. The selected places were: Chittagong (a sea-port town in the extreme south-east), Dibrugarh (a large town in the extreme north-east), Jatrabari (a small village east of Dacca), and Raibazaar (a large village near Dacca).

The visits to these places were begun early in February when it became possible to leave Dacca, the work there having been so organised that it could be carried on efficiently during our temporary absence.

[1] The rats caught in these special traps were excluded in calculating the number of rats per 100 traps set throughout the year.

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Each place was visited for a period of a week or ten days according to its size. A short period was selected to enable us to visit a number of places. This plan we considered to be better than devoting a longer period to fewer places for we were always able to compare the results obtained during our short visits (although the figures were small) with the prolonged observations in Dacca. We took with us on each visit from 40 to 70 traps according to the size of the place to be visited. The town or village having been divided up into a number of sections, batches of from eight to ten traps were allotted to each section so that even in so short a period as ten days a fair sample of the rats and their fleas for that particular time of the year was obtained.

(*b*) *The work in the neighbouring plague area of Bengal.* A reference to the map and Table I will show that, with the exception of Calcutta and two districts immediately adjoining it, practically all the plague has been confined to a comparatively small area comprising some eight to ten districts in the north-western portion of the province of Bengal. This latter area is roughly triangular in shape, with its base abutting on the United Provinces while its apex lies in the Bhagalpur district. A further examination of the distribution of the disease in this area shows that its greatest intensity is in the immediate neighbourhood of the Ganges river. Thus, while the district of Saran has an average annual plague death rate for the seven year period above mentioned of 77 per 10,000 of the population, the adjoining district of Champaran has only a plague death rate of 0·3 per 10,000 for the same period. Again in the Bhagalpur district practically all the plague occurs in the two or three "thanas" or subdivisions which are nearest the river, that is, in the centre of the district.

The monthly distribution of plague deaths in Bengal and Calcutta city is shown separately in Tables II and III for the years 1901 to 1909 in the case of Bengal, and for 1900 to 1909 in the case of Calcutta. Charts I and II graphically demonstrate the seasonal prevalence of plague in Bengal and Calcutta respectively.

Of the various towns in the triangular plague area of Bengal, the largest one, nearest to the border of Eastern Bengal, which had been visited by plague epidemics in several previous years, was Bhagalpur, the capital town of the Bhagalpur district in Behar. We therefore determined, after our visit to Chittagong, to proceed to Bhagalpur, to compare the conditions found there with those observed in Eastern Bengal. Bhagalpur had the advantage too that at the time of our visit plague was absent from the town nor had the disease been prevalent

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for the past two years. We obtained results therefore, as far as rat and flea infestation was concerned, which more nearly approximated to the normal than had the town recently suffered from the disease.

TABLE I.

Showing the total and the mean plague death rate per mille, in the different districts of the Province of Bengal (except Calcutta city) for seven years from 1903 *to* 1909 *(inclusive).*

Names of districts	Population according to census of 1901	Total plague deaths from 1903 to 1909 (inclusive)	Total plague death-rate per mille from 1903 to 1909 (inclusive)	Mean plague death-rate per mille from 1903 to 1909 (inclusive), 7 years
Burdwan	1,532,475	144	0·094	0·013
Birbhum	902,280	3	0·003	0·0004
Bankura	1,116,411	7	0·006	0·0009
Midnapore	2,789,114	33	0·012	0·002
Hooghly	1,049,282	520	0·496	0·071
Howrah	850,514	1882	2·213	0·316
24 Parganas	2,078,359	544	0·262	0·037
Nadia	1,667,491	112	0·067	0·010
Murshidabad	1,333,184	4	0·003	0·0004
Jessore	1,813,155	8	0·004	0·0006
Khulna	1,253,043	7	0·005	0·0007
Patna	1,624,985	102,022	62·783	8·969
Gaya	2,059,933	39,166	19·013	2·716
Shahabad	1,962,696	52,866	26·935	3·848
Saran	2,409,509	130,161	54·020	7·717
Champaran	1,790,463	414	0·231	0·033
Muzaffarpur	2,754,790	18,574	6·742	0·963
Darbhanga	2,912,611	20,806	7·143	1·020
Monghyr	2,068,804	30,392	14·691	2·099
Bhagalpur	2,088,953	7514	3·597	0·514
Purneah	1,874,794	16	0·008	0·001
Darjeeling	249,117	12	0·048	0·007
Sonthal Parganas	1,809,737	131	0·072	0·010
Cuttack	2,062,758	116	0·056	0·008
Balasore	1,071,197	4	0·004	0·0006
Puri	1,017,284	Nil	Nil	Nil
Hazaribagh	1,177,961	676	0·574	0·082
Ranghi	1,187,925	2	0·002	0·0003
Palaman	619,600	1096	1·769	0·253
Manbhum	1,301,364	37	0·028	0·004
Suighbhum	613,579	4	0·003	0·0004

30,346 deaths from plague took place in Calcutta city from 1903 to 1909 (inclusive). Total plague death rate per mille for the period is 35·794 and the mean plague death rate is 5·11.

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TABLE II.

Showing the monthly figures of plague deaths in the districts of Bengal, including towns, from 1901 *to* 1909 *(both years inclusive). The plague deaths of Calcutta city have been excluded.*

	1901	1902	1903	1904	1905	1906	1907	1908	1909	Total plague deaths, past 9 years (1901 to 1909), both years inclusive	Average monthly plague deaths, past 9 years (1901 to 1909), both years inclusive
January	9034	2725	9518	8320	20,682	5316	5426	1522	799	63,342	7038·0
February	14,131	3821	11,750	13,851	22,472	10,212	10,847	3267	1056	91,407	10,156·3
March	26,976	5756	17,449	21,958	38,037	19,197	23,524	5593	1773	160,263	17,807·0
April	15,651	3536	9387	8881	23,418	15,561	29,420	2565	702	109,121	12,124·6
May	2120	1053	952	1375	7825	1451	7171	181	300	22,428	2492·0
June	140	135	108	536	1352	189	696	26	77	3259	362·1
July	18	40	62	480	92	60	245	11	55	1063	118·1
August	47	20	50	1785	198	386	424	16	124	3050	338·9
September	271	92	205	1659	340	533	453	66	180	3799	422·1
October	350	544	541	1011	485	476	279	126	230	4042	449·1
November	627	1749	2151	2166	1169	851	393	287	903	10,296	1144·0
December	1381	6218	5285	8725	2642	2781	1133	509	3026	31,700	3522·2
Yearly total	70,746	25,689	57,458	70,747	118,712	57,013	80,011	14,169	9225	503,770	55,974·4

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TABLE III.

Showing the monthly plague deaths in Calcutta city from 1900 *to* 1909 *(both years inclusive).*
Figures obtained from the reports of Sanitary Commissioner.

Months	1900	1901	1902	1903	1904	1905	1906	1907	1908	1909	Total plague deaths, past ten years (1900 to 1909), both years inclusive	Average monthly plague deaths, past 10 years (1900 to 1909), both years inclusive
January	218	283	169	260	73	186	103	60	58	64	1474	147·4
February	653	703	506	1058	290	408	124	89	97	84	4012	401·2
March	3531	3944	2282	3140	1170	1503	684	454	341	294	17,343	1734·3
April	1855	2011	2457	2801	2293	3509	935	1420	501	386	18,168	1816·8
May	672	416	1266	499	529	1093	306	972	364	717	6834	683·4
June	451	174	248	71	159	198	190	283	130	273	2177	217·7
July	230	58	78	47	44	58	36	63	91	138	843	84·3
August	229	91	69	56	19	48	44	26	67	53	702	70·2
September	229	60	45	74	12	70	38	30	32	28	618	61·8
October	90	66	35	83	12	24	37	30	40	36	453	45·3
November	9	107	45	78	31	74	47	82	28	21	522	52·2
December	108	97	78	57	71	101	63	80	32	23	710	71·0
Yearly total	8275	8010	7278	8224	4703	7272	2607	3589	1781	2117	53,856	5385·6

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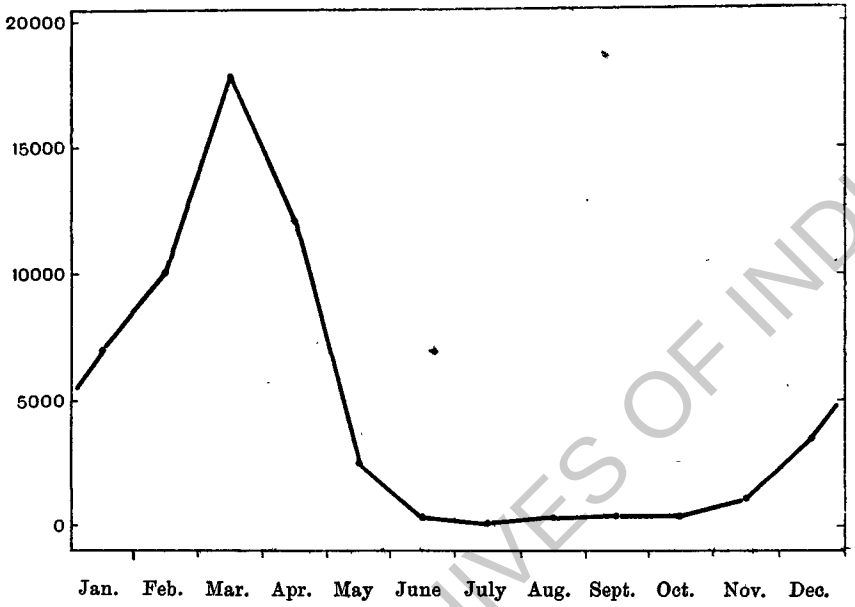

Chart I. Seasonal prevalence of human plague in Bengal exclusive of Calcutta.

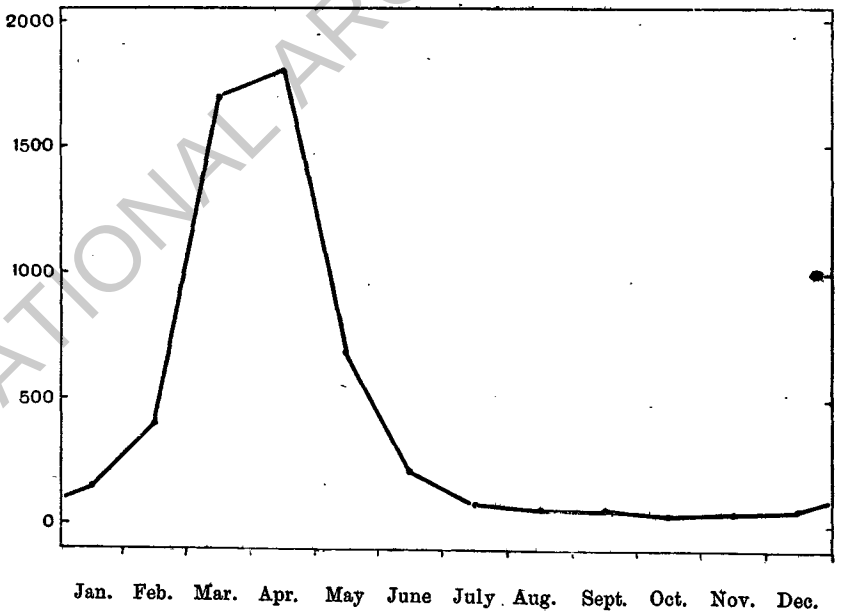

Chart II. Seasonal prevalence of plague in Calcutta.

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When the work in Bhagalpur had been completed we found that the conditions there differed so remarkably from those observed in Eastern Bengal that we determined to examine another town. We decided to select one which had been free from plague but which was situated between the boundary of Eastern Bengal and Bhagalpur; our choice fell on Purneah.

D. Facilities for the Importation of Infection and for the spread of Infection from one place to another within the Province.

(1) *Physical features of the country.*

The province of Eastern Bengal and Assam has an area of 111,569 square miles, and in shape is roughly quadrangular (see map).

On the north it is bounded by the Himalayan mountains. On the east it is separated from Burmah by an almost equally inaccessible series of mountains covered with dense forests, and on the south it is limited by the Bay of Bengal. The western portion of the province adjoins Bengal proper, but is separated from Bengal along the greater part of this western boundary by the Ganges river.

Projecting to the plain from the hilly eastern border is the range of the Khasi and Garo hills covered with dense jungle. These hills almost completely divide Assam in the north from Eastern Bengal in the south. With the exception of these hills and certain other hilly tracts the major part of the province consists of the rich fertile and low-lying alluvial valleys of the Brahmaputra and Ganges rivers, with their many tributaries.

It will thus be seen that impenetrable mountain ranges practically limit the importation of infection from the north and east while the sea on the south and the Ganges river on the west greatly curtail the opportunities for the importation of infection in these directions.

(2) *Propinquity to infected places.*

We have already referred to the plague-infected areas in Bengal. We pointed out that one area existed in Calcutta and its neighbourhood while another was to be found in the north-western portion of that province. These plague-infected areas are both separated from the

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province of Eastern Bengal and Assam by a considerable tract of relatively plague-free country. Thus the Calcutta area is separated from Eastern Bengal and Assam by the plague-free districts of Nadia, Jessore, Khulna, and 24 Paganas; while between the other plague area and Eastern Bengal the plague-free districts of Purneah and Sonthal Parganas intervene.

(3) *Communication with infected places by rail, road and water.*

There are two main systems of railway which carry traffic from plague-infected areas into Eastern Bengal and Assam. The Eastern Bengal State Railway passes from west to east along the northern portion of the province and carries traffic from the north-western plague-infected portion of Bengal into the province. The Assam-Bengal Railway carries traffic from the port of Chittagong in the south of the province in a northerly direction to Dibrugarh. This railway is the means by which a large trade with Calcutta is maintained. These two main railway systems are developing rapidly, but direct communication along them is frequently interrupted especially in the deltaic area of the Ganges and Brahmaputra. The numerous mouths of those large rivers with their shifting beds render bridge building practically impossible.

There are very few good roads in Eastern Bengal, especially in the southern parts of the province. This is probably owing to the frequent flooding of the low-lying country, the sandy nature of the soil, the scarcity of stone and the many intersecting streams which often change their course.

Navigable rivers and canals are numerous and afford the chief means of communication between one place and another within the province. Travelling by this means although cheap is yet very slow. Grain conveyed from Calcutta to, for instance, Dibrugarh would take several weeks to reach its destination by country boat and more than a week by steamer. It is important to bear this in mind in connection with the possible conveyance of infection by this channel. Owing to the melting of the winter snows in the Himalayas and the heavy rainfall during the six months May to October, the rivers and canals overflow and flood the low-lying country which, as we have seen, constitutes the major part of the province. Communication between village and village during this season is greatly interrupted. The floods

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are generally so extensive as to prevent easy communication even between one tenement and another in the same village. Some photographs showing the flooding which takes place during the rainy season are here reproduced (figs. 1 and 2). In one of them a man may be seen making his way over the flooded fields in a large earthenware vessel, a common mode of progression during this season.

(4) *Extent and nature of the trade especially with plague centres.*

The population of the province in 1901 was 30,961,459. The density of the population, especially in the fertile villages, is very great and frequently lies between 950 to 500 per square mile; yet large towns and villages are few. Only 2 % of the population were enumerated in the 61 towns of the province. The villages are generally made up of a number of hamlets scattered over the plain. These hamlets are situated on slight elevations and are surrounded on all sides by low-lying land which, as we have seen, for several months of the year is covered with water (see fig. 3).

Agriculture is the chief occupation of the people. The land is fertile and yields crops readily. Famines are unknown. The population is therefore generally well-to-do; extreme poverty such as is common in other parts of India is seldom met with. The staple food crop is rice which is produced in sufficient quantity to meet the requirements of the inhabitants; 60 % of the total cropped area is covered with this cereal. In Assam, however, large areas are occupied by tea gardens. The labourers on these gardens come from many parts of India outside the province. A large and ever increasing cosmopolitan population is thus springing up in this part. The habits and food supply of these people differ from those of the indigenous inhabitants, and this is an important point to remember in connection with the importation of plague infection. One of the largest towns in this tea area is Dibrugarh in the extreme north-east of the province which, as we have seen, has passed through one mild epidemic of plague.

Very little grain is imported into the province since most of that which is consumed is grown locally. The chief imports are cotton piece goods, gunny bags (new), salt, sugar, kerosine oil, etc.—materials in fact which are little likely to carry plague infection in the form of infected rats or rat fleas.

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E. Climate of Eastern Bengal and Assam.

It is difficult to give any general account of the climatic conditions in a province like Eastern Bengal and Assam, the hilly tracts differing so remarkably from the low valleys. Roughly, however, the province, from a climatological point of view, may be divided into Assam in the north and Eastern Bengal in the south.

(*a*) The mean *temperature* in the northern portion of the province seldom rises above 80° F. except in July, August and September and is generally below 75° F. for seven months in the year. In the southern portion of the province the mean temperature remains more or less above 80° F. from April till October. The diurnal variation of the temperature is small, being about twenty degrees during the winter months, fifteen degrees in April, May, June, October and November, while from July to September there is only a diurnal variation of about ten degrees.

(*b*) The relative *humidity* in Assam never falls below a monthly mean of 80 % of saturation, but in Eastern Bengal considered as a whole the monthly mean humidity is below 80 % from January to April.

(*c*) The *rainfall* in the northern portion of the province averages about one hundred inches in the year but the rainfall differs remarkably in the hills and plains. At Cherrapungi for example the annual rainfall averages four hundred and fifty-eight inches (nine hundred and five inches are said to have fallen in 1861) while at Lanka in the Kapli valley in Nowgong the average fall is less than forty-three inches. The greatest precipitation occurs in the months May to October, with an average of over ten inches during each of these months. In the southern portion of the province the average rainfall is about seventy-five inches, the greatest precipitation occurring from May to September.

The climate of Eastern Bengal and Assam is thus characterised by coolness and extreme humidity, the natural result of the great water surface over which evaporation takes place and the close proximity of hill ranges on which an excessive precipitation occurs. Its most distinguishing feature is the copious rainfall between March and May, a time when precipitation over the rest of India is at its minimum.

Table IV gives the normal temperature and humidity calculated from readings made at Narayanganj Observatory near Dacca town. The table also shows the calculated average mean monthly temperature and 8 a.m. humidity for the year of our observations, 1910.

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TABLE IV.

Showing the normal monthly mean temperatures and 8 *a.m. humidity as well as mean monthly temperatures and* 8 *a.m. humidity for* 1910 *at Narayanganj.*

Month	Normal				1910			
	Mean max. temp.	Mean min. temp.	Mean of max. and min. temp.	Mean humidity at 8 a.m.	Mean max. temp.	Mean min. temp.	Mean of max. and min. temp.	Mean humidity at 8 a.m.
January	77·9°	55·2°	66·5°	87 %	75·4°	54·3°	64·9°	83 %
February	82·2	58·6	70·4	84	81·7	57·6	69·6	79
March	90·0	68·2	79·1	83	88·0	67·6	77·8	79
April	93·2	74·3	83·7	82	91·9	73·9	82·9	81
May	91·3	75·8	83·5	85	91·2	75·5	83·4	83
June	89·0	78·3	83·7	89	88·7	78·0	83·3	89
July	88·3	79·2	83·7	90	87·4	77·8	82·6	89
August	87·5	79·0	83·3	90	87·9	78·9	83·4	88
September	88·5	78·9	83·7	88	89·0	79·0	84·0	86
October	87·9	75·3	81·6	84	88·5	77·5	83·0	87
November	83·5	66·0	74·7	85	83·0	65·0	74·0	78
December	78·0	57·3	67·7	87	76·8	56·2	66·5	83

F. An Examination of the Rats and their Fleas found in Houses.

(I) *Data collected in Dacca town from* 13*th December,* 1909 *to* 31*st December,* 1910.

(*a*) *Rats.*

(1) If we include musk rats in the category of rats, then four species were found in Dacca houses. They were present in the following proportions:

Species	Number of rats	Proportion %
M. rattus	5704	48·5
Gunomys varius	2064	17·6
Musk rats	2337	19·9
Mice	1650	14·0

In all a total of 11,755 rats were caught and examined in Dacca town. Of these 5704 were *M. rattus* and among them 595 belonged to that variety with a white belly which we have occasionally referred to in our previous reports as *Mus rattus* var. *alexandrinus.*

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It is of interest to compare the proportion of *M. rattus* to other rats caught in Dacca with similar figures obtained in Poona, Belgaum, Bombay and Madras, thus:

Place	*M. rattus* to all rats	Other kinds of rats
Dacca	48·5 %	Many *G. varius*, musk rats and mice found.
Poona	98·6	A few musk rats and mice.
Belgaum	95·7	A few musk rats, mice and bandicoots.
Bombay	66·2	Many *M. decumanus*, a few musk rats, mice and *G. varius*.
Madras	49·4	Many mice 46·1 %, a few musk rats and bandicoots.

The proportion of rats caught to traps set is shown month by month in Table V. In Chart III a contrast is drawn between the number of rats caught per month per 100 traps set in Dacca with similar figures obtained during the same period in Poona city. It is here necessary to state that, from the time (January, 1910) when the Poona record shown in the chart begins, plague had been absent from the city for one year and eight months. During this plague-free period in Poona the rats caught per 100 traps set had steadily risen from sixteen rats to approximately forty. It will be seen from the chart that the number of rats caught per 100 traps set still continued to rise during the year 1910, in the complete absence of plague, to approximately seventy-five

TABLE V.

Showing the number of Mus rattus, Gunomys varius, *mice and musk rats per* 100 *traps set, in monthly periods. Dacca,* 1909—1910.

Period	Traps set	Rats caught				Rats per 100 traps set			
		Mus rattus	*G. varius*	Mice	Musk rats	*Mus rattus*	*G. varius*	Mice	Musk rats
December 13th to 31st, 1909	2945	274	63	240	87	9·3	2·1	8·1	3·0
January 1910	5334	391	95	281	149	7·3	1·8	5·3	2·8
February ,,	4953	431	60	113	142	8·7	1·2	2·3	1·1
March ,,	7491	490	170	80	105	6·5	2·3	1·1	1·4
April ,,	7153	348	154	77	139	4·9	2·2	1·1	2·4
May ,,	7225	422	131	78	177	5·8	1·8	1·1	2·4
June ,,	7436	483	200	93	153	6·5	2·7	1·3	2·1
July ,,	7151	433	129	66	242	6·1	1·8	0·9	3·4
August ,,	6068	370	219	52	169	6·1	3·6	0·9	2·8
September ,,	7074	486	169	44	165	6·9	2·4	0·6	2·3
October ,,	6143	468	212	115	182	7·6	3·5	1·9	3·0
November ,,	6792	621	246	162	303	9·1	3·6	2·4	4·5
December ,,	6723	487	216	222	317	7·2	3·2	3·3	4·7

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rats for every 100 traps set. The chart clearly shows that very few rats were caught in Dacca in proportion to the number of traps set, and contrasts this experience with that observed by us in other parts of India, especially in such a plague-infected city as Poona.

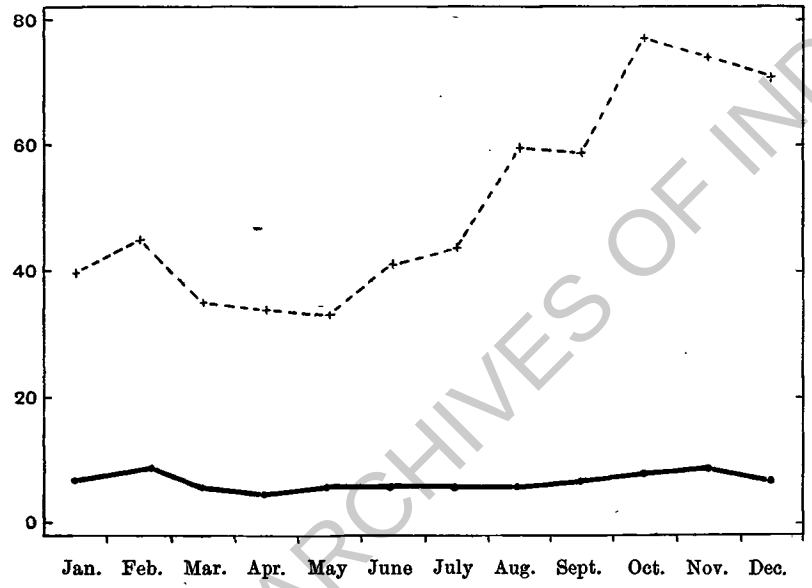

Chart III. *Rattus* caught per 100 traps in Dacca (•——•), and in Poona (+-----+) in 1910.

(2) *Seasonal variation in the number of rats caught in Dacca.*

Figures bearing on this matter are to be seen in Table V where it will be noted that, except in the case of mice, there is not much seasonal variation in the number of rodents caught in the houses; there is however a tendency for all kinds of rats to be more readily caught in houses during the cold weather, an observation which accords with our experience in other parts of India where we have worked.

(3) *Breeding season of* Mus rattus *in Dacca.*

Statistics in this connection are shown in Tables VI and VII and these are graphically depicted in Chart IV. It is evident that although breeding takes place all the year round, the greatest number of adult females pregnant is found in the months of February, March, April and

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May. This increase in the number of pregnant females is followed in the months of March, April, May, June and July by an increase in the number of young rats caught. The breeding season among rats here therefore is most active during the hot weather and this observation is in harmony with our experience in other parts of India (Vol. VII. pp. 749, 906; X. pp. 457, 520).

TABLE VI.

Showing the percentage of adult female rattus *pregnant in monthly periods. Dacca.*

Period	Adult female *rattus*	Adult females pregnant	Percentage of adult females pregnant
December 13th to 31st 1909	97	19	19·6
January 1910 ...	163	29	17·8
February ,, ...	171	61	35·7
March ,, ...	159	62	39·0
April ,, ...	83	28	33·7
May ,, ...	136	59	43·4
June ,, ...	151	44	29·1
July ,, ...	151	45	29·8
August ,, ...	144	43	29·9
September ,, ...	205	64	31·2
October ,, ...	179	50	27·9
November ,, ...	218	53	24·3
December ,, ...	184	38	20·7

TABLE VII.

Showing the percentage of young rattus *in monthly periods. Dacca.*

Period	*Rattus* weighed	Young *rattus*	Percentage of young *rattus*
December 13th to 31st 1909	274	86	31·4
January 1910 ...	391	109	27·9
February ,, ...	431	128	29·7
March ,, ...	490	223	45·5
April ,, ...	348	187	53·7
May ,, ...	422	187	44·3
June ,, ...	483	210	43·6
July ,, ...	433	176	40·6
August ,, ...	370	112	30·3
September ,, ...	486	187	38·5
October ,, ...	468	154	32·9
November ,, ...	621	228	36·7
December ,, ...	487	130	26·7

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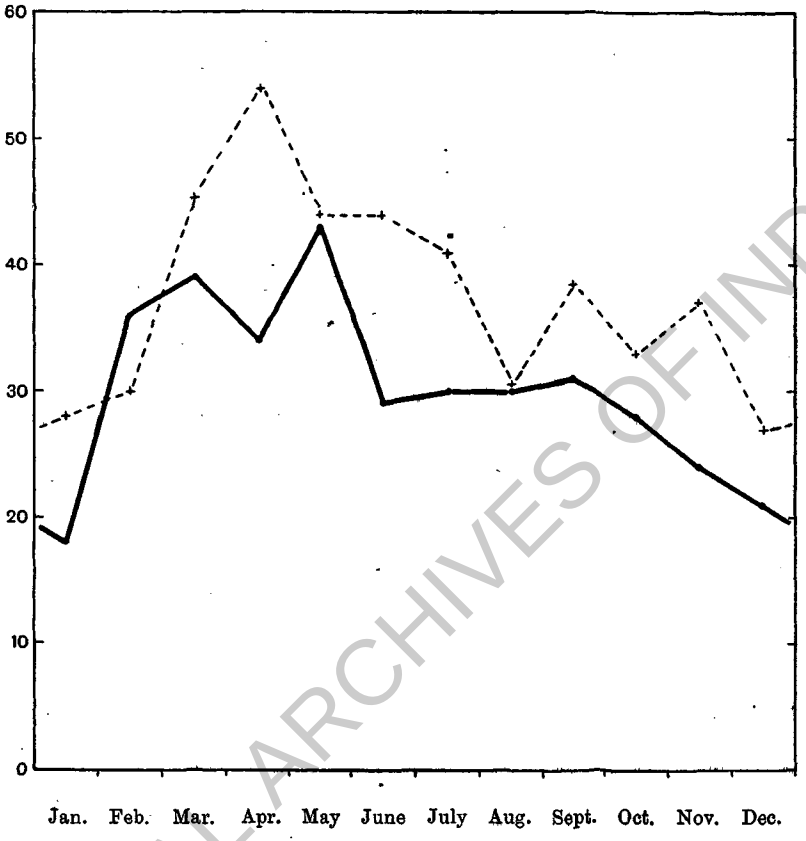

Chart IV. Percentage of female rats pregnant (•——•).
Percentage of young rats (+-----+).

(4) *Other observations bearing on the habits of rats.*

We have already mentioned that a certain number of traps were set with the object of ascertaining the comparative rat infestation of different types of houses and again that other traps were set with the object of testing the efficiency of different kinds of baits. The material collected in this way is displayed in Tables VIII to XII. A few remarks are here necessary to explain these tables. The words "pucca" and "kutcha," when applied to buildings, are commonly used in India to indicate on the one hand a well-made, solid brick and mortar or stone

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building and on the other hand a flimsy cheap shelter of mud or bamboo or palm matting.

In Table VIII figures are given which show the result of trapping buildings according as they were regarded as "pucca houses or shops," "kutcha houses or shops," "godowns (store houses) or stables." This table shows that apart from godowns or stables more *M. rattus* were taken in well-built houses than in "kutcha" houses. It is interesting to note that this experience is exactly reversed in the case of musk rats and suggests the possibility that the one species is inimical to the other. Experiments have shown that the musk rat fiercely attacks and kills *M. rattus* when the two species are confined together in a cage. This is a matter of no little importance when we remember that the musk rat rarely if ever suffers from plague (Vol. VII. pp. 760, 904; X. p. 456).

TABLE VIII.

Showing the number of Mus rattus, *mice, musk rats and* G. varius *per* 100 *traps set, arranged according to the nature of house, shop or godown. Dacca.*

Three series: Jan. 9 to 30, 1910, June 20 to July 17, 1910, Oct. 10 to Nov. 6, 1910.

Nature of house, shop or godown	Traps set	Rats caught				Rats per 100 traps set			
		Rattus	Mice	Musk rats	*G. varius*	*Rattus*	Mice	Musk rats	*G. varius*
Pucca house or shops	9741	865	303	290	329	8·9	3·1	3·0	3·4
Kutcha ,, ,,	7554	245	126	242	125	3·2	1·7	4·5	1·7
Others, including godowns and stables	183	31	3	5	16	16·9	1·6	2·7	8·7

TABLE IX.

Showing the number of Mus rattus, *mice, musk rats and* G. varius *per* 100 *traps set in houses arranged according to the nature of the roof of the houses. Dacca.*

Three series combined: Jan. 31 to March 6, 1910, July 18 to Aug. 14, 1910, Nov. 7 to Dec. 4, 1910.

Nature of roof	Traps set	Rats caught				Rats per 100 traps set			
		Rattus	Mice	Musk rats	*G. varius*	*Rattus*	Mice	Musk rats	*G. varius*
Masonry roof	12,498	1046	224	437	357	8·4	1·8	3·5	2·9
Tin ,,	5358	325	126	195	66	6·1	2·4	3·6	1·2
Thatch ,,	1407	55	31	51	27	3·9	2·2	3·6	1·9
Other ,,	2	0	0	0	0	0	0	0	0

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Table IX gives the result of trapping when regard is paid to the character of the roof of the buildings in which the traps are set. Here it appears that buildings with masonry roofs harboured the greatest proportion of rats, that the next largest proportion were caught in buildings with "tin" or corrugated iron roofs, and that buildings with roofs made of thatch or matting sheltered fewest rats. Tables X and XI give the results of trapping when regard is paid to the nature of the walls and floors of the buildings trapped.

TABLE X.

Showing the number of Mus rattus, *mice, musk rats and* G. varius *per* 100 *traps in houses arranged according to the nature of wall. Dacca.*

Four series: Dec. 5, 1909 to Jan. 31, 1910, March 7 to April 24, 1910, April 25 to May 22, 1910, August 15 to Sept. 11, 1910.

Nature of wall	Traps set	Rats caught				Rats per 100 traps set			
		Rattus	Mice	Musk rats	*G. varius*	*Rattus*	Mice	Musk rats	*G. varius*
Brick wall	19,279	1437	296	469	637	7·5	1·5	2·4	3·3
Mud	4619	183	49	83	27	4·0	1·1	1·8	0·6
Chattac ,,	3059	149	43	72	55	4·9	1·4	2·4	1·8
Other ,,	2297	48	29	74	40	2·1	1·3	3·2	1·7

TABLE XI.

Showing the number of rats per 100 *traps set, arranged according to the nature of the floor. Dacca.*

Two Series: May 29 to June 19, 1910, Sept. 12 to Oct. 9, 1910.

Nature of floor	Traps set	Rats caught				Rats per 100 traps set			
		M. rattus	Mice	Musk rats	*G. varius*	*M. rattus*	Mice	Musk rats	*G. varius*
Pucca	7981	696	92	214	224	8·7	1·2	2·7	2·8
Kutcha	5173	222	44	106	96	4·3	0·8	2·0	1·8

These results, however, do not give a true indication of the efficiency of the particular materials used in the construction of the buildings in preventing the ingress of rats or of affording shelter or protection to them; other factors of greater importance, such as the situation of the buildings, the habits of the people dwelling in the houses, the size of the buildings[1], the nature of the furniture and the other materials

[1] "Pucca" are generally considerably larger than "kutcha" houses.

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stored in them, all play some, or even a greater part, in encouraging or discouraging the presence of rats in houses. A well-constructed house is not of itself a safeguard against rats although other conditions remaining constant a "pucca" house must of necessity be better than a "kutcha" one in this respect. Our figures simply serve to show once again that a well-constructed house in itself is not a complete protection against rat infestation.

Table XII gives the results of tests to ascertain which of a number of baits were most effective in attracting rats. It will be seen that bread and plantain are more efficient as baits for *Mus rattus* than cocoa-nut or dried fish.

TABLE XII.

Showing the average number of Mus rattus, *mice, musk rats and* G. varius *per* 100 *traps set according to the nature of bait.*

Kind of bait	Traps set	Rats caught				Rats per 100 traps set			
		M. rattus	Mice	Musk rats	*G. varius*	*M. rattus*	Mice	Musk rats	*G. varius*
Plantain	2012	161	48	44	42	8·0	2·4	2·2	2·1
Bread	21,090	1527	457	709	485	7·2	2·2	3·4	2·3
Cocoa-nut	1851	93	34	53	28	5·0	1·8	2·8	1·5
Bread	19,736	1392	330	607	547	7·1	1·7	3·1	2·7
Dry fish	1833	61	27	47	68	3·3	1·5	2·6	3·7
Bread	18,973	1254	240	552	545	6·6	1·3	2·9	2·9

(5) *Pathological lesions met with in rats in Dacca.*

The only pathological lesions in rats that were of particular interest to us were lymphatic and splenic abscesses and signs of old perisplenitis, adhesions, and scars in the spleen. The presence of these conditions was carefully noted when met with in order to compare their frequency with similar conditions observed in Poona, Bombay and Belgaum where (Vol. x. p. 335) similar lesions among rats were attributed to epizootic plague. Our findings as regards Dacca are summarised in Table XIII. We may remark in connection with this table that almost all the lesions recorded could, to a trained eye, in the light of our subsequent experience, be distinguished from true resolving plague lesions. We do not intend here to enter into the differences between the lesions found in Dacca and those noted in Poona and Belgaum but merely record the fact that appearances may be met with in rats which have not been

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exposed to plague which, to the unskilled observer, may be mistaken for resolving plague lesions. The former appear to exhibit some seasonal variation in frequency.

TABLE XIII.

Showing the percentage of adhesions, scars, perisplenitis, and peripheral and visceral abscesses in Mus rattus *for the year* 1910 *in quarterly periods. Dacca, Jan.* 1*st to Dec.* 31*st,* 1910.

Quarter ending		*M. rattus* weighed	Numbers of					Percentage				
			Adhesions	Scars	Perisplenitis	Peripheral abscesses	Visceral splenic abscesses	Adhesions	Scars	Perisplenitis	Peripheral abscesses	Visceral splenic abscesses
March	1910	1312	5	12	11	5	1	0·38	0·91	0·84	0·38	0·08
June	,,	1253	3	14	4	2	1	0·24	1·12	0·32	0·16	0·08
September	,,	1289	2	13	5	2	1	0·16	1·01	0·39	0·16	0·08
December	,,	1576	3	17	5	1	Nil	0·19	1·08	0·32	0·06	Nil

TABLE XIV.

Showing the average number of fleas per G. varius, *mice, musk rats, and* Mus rattus *in monthly periods. Dacca,* 1909—1910.

Monthly periods		*G. varius*			Mice			Musk rats			*M. rattus*		
		Flea counts made	Total fleas	Average fleas	Flea counts made	Total fleas	Average fleas	Flea counts made	Total fleas	Average fleas	Flea counts made	Total fleas	Average fleas
Dec. 13th to 31st,	1909	59	45	0·8	231	28	0·1	77	47	0·6	257	660	2·5
January	1910	93	167	0·8	271	69	0·3	70	58	0·8	388	1235	3·2
February	,,	57	209	3·7	96	19	0·2	28	15	0·5	421	2088	5·0
March	,,	166	732	4·4	71	25	0·4	23	8	0·3	464	2576	5·6
April	,,	150	695	4·6	70	33	0·5	19	46	2·4	339	2777	8·2
May	,,	118	599	5·1	71	45	0·6	30	23	0·8	402	2861	7·1
June	,,	195	1306	6·7	90	34	0·4	23	10	0·4	445	2655	6·0
July	,,	118	731	6·2	65	26	0·4	50	50	1·0	415	1929	4·6
August	,,	207	783	3·8	49	19	0·4	39	27	0·7	355	1213	3·4
September	,,	164	331	2·0	39	19	0·5	30	14	0·5	466	1092	2·3
October	,,	189	264	1·4	95	33	0·3	43	25	0·6	430	740	1·7
November	,,	203	604	3·0	135	44	0·3	37	20	0·5	573	1667	2·9
December	,,	183	454	2·5	188	27	0·1	31	6	0·2	442	1210	2·7
Total		1902	6920	3·6	1471	421	0·3	500	349	0·7	5397	22,703	4·2

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(b) Rat fleas.

(1) With the exception of a few human and cat fleas caught on rats in Dacca town, all the fleas found were *X. cheopis*. Of the four varieties of rats (including for the sake of convenience the musk rat) found in Dacca only two, viz. *M. rattus* and *G. varius*, harboured this flea in any numbers. The mouse and musk rat carried very few fleas. Table XIV gives the average number found on each species of rat in monthly periods.

(2) Considering only the fleas found on *M. rattus* a very distinct seasonal prevalence was observed. This seasonal variation has been graphically depicted in Chart V. It will be seen that the lowest average number of fleas was found in October, when two per rat were recorded. From October onwards to April the average number of fleas per rat increased to approximately eight. The greatest number of fleas was

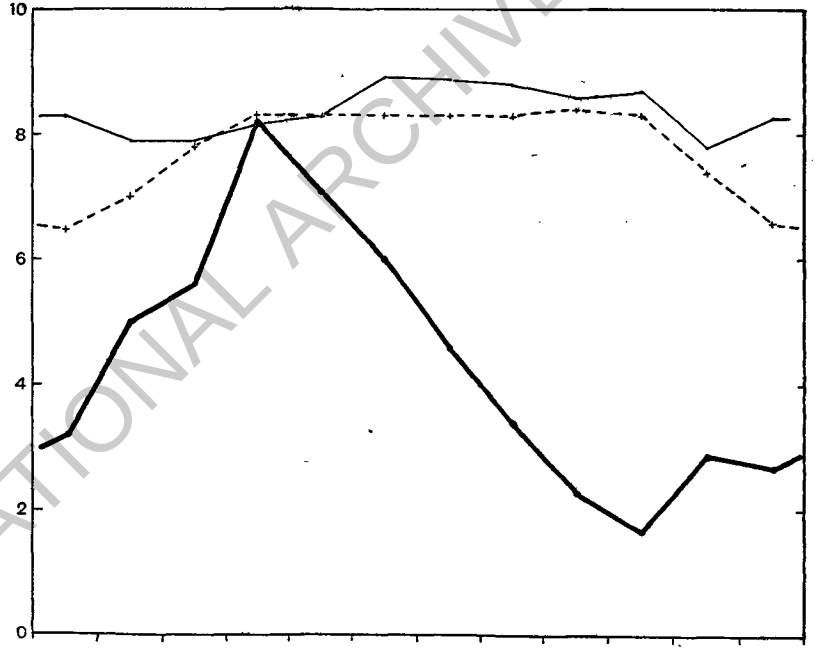

Chart V. Seasonal prevalence of rat fleas in Dacca (•——•).
Mean monthly temperature (+- - - - -+).
Mean monthly humidity (•——•).

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noted in the week ending 10th April when thirteen fleas per rat were recorded. Thereafter a steady decline in the number of fleas occurred till the month of October. It is interesting to note that the curve of the seasonal prevalence of fleas in Dacca (Chart V) corresponds fairly well with the curve of the seasonal prevalence of plague in Calcutta (Chart II).

(II) *Data collected in the district.*

The towns and villages were visited in the following order:—(1) Chittagong, (2) Jatrabari (first trapping), (3) Bhagalpur, (4) Purneah, (5) Raibazaar, (6) Dibrugarh, (7) Jatrabari (second trapping). With the exception of the second trapping of Jatrabari village these places were all visited during the early part of the year 1910 (February to April inclusive), *i.e.* at a time corresponding with the plague season in Bengal. It may be presumed therefore, that, considering the Dacca results, flea prevalence was not below the average at the time each place was examined. Jatrabari village was visited however a second time during the rainy season when the whole surrounding country was under water. This visit was made to find out whether the floods which then covered the country had driven rats into the houses and to see whether we could capture more rats in the houses during the rainy season.

(1) *Chittagong.* (Figs. 19, 20.) This town is an important sea-port in the south-eastern portion of the province situated some ten miles up the Karnapauli river. It has a population of 22,140 who live scattered over a considerable area. It is connected with Assam by the main line of the Assam-Bengal railway. Shipping is carried on chiefly with Burmah and Calcutta. Cargo ships come alongside the jetty while passenger ships tie up as a rule in mid stream. In the native city the houses or groups of houses are very scattered. In the middle of the town there are a number of small hills upon which stand the principal offices and the bungalows of the chief European inhabitants. Trapping commenced in the town on the 4th February on the plan described above (p. 162 f.) and was continued till the 12th of the same month with the following results.

Rats caught:

M. rattus	Mice	Musk rats	*G. varius*
65	87	23	45

Average fleas per *M. rattus*	...	...	3·8
Number of traps set	...	...	620
All rats per 100 traps set	...	..	35·5
M. rattus per 100 traps set	...	..	10·5

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(2) *Jatrabari village.* (Figs. 3, 4.) This village is situated on a fairly good road about four miles distant from Dacca town. It was selected as a typical example of a Bengali village. It consists of a long strip of tenements arranged in the shape of the letter **S**, intersected along the middle by the main road; the houses are built on mounds of earth raised above the surrounding country which for a considerable portion of the year is more or less under water. The inhabitants numbered 1325. Trapping commenced on the 18th February and was continued for eight days till the 25th with the following results.

Rats caught:

M. rattus	Mice	Musk rats	*G. varius*
6	46	9	0

Average fleas per *M. rattus*	2·3
Number of traps set	320
All rats per 100 traps set	19·1
M. rattus per 100 traps set	1·9

The village was trapped a second time from the 18th to the 23rd August. At this time the whole of the surrounding country was under water.

Rats caught:

M. rattus	Mice	Musk rats	*G. varius*
15	23	9	4

Average fleas per *M. rattus*	0·3
Number of traps set	359
All rats per 100 traps set	14·2
M. rattus per 100 traps set	4·2

(3) *Bhagalpur.* (Figs. 15–18.) With the object of comparing the trapping in Dacca, Chittagong and Jatrabari, which places had never suffered from plague, with a town which had suffered from the disease and which was nearest to Eastern Bengal (Calcutta excepted[1]) a visit was paid to Bhagalpur. This is a large town in Behar. It is situated on the right bank of the river Ganges and has a population of 75,760. It stretches for a length of about six miles and is made up of three portions. It is in railway communication with Calcutta on the one side and the plague-stricken area of Patna and Ballia on the other. Good road and river communication exists in addition between these places. Except for the last two years (1909–1910) epidemics of plague,

[1] Calcutta was not visited because other conditions such as overcrowding, foreign population, etc. come into play. It is an exceptional rather than a representative town for this part of India.

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although by no means so severe as in many towns of India, have repeatedly visited the place. The average annual plague mortality for the last ten years per 1000 of population has been 5·5. Trapping commenced on the 27th February and was continued for ten days till the 8th March with the following results.

Rats caught :

M. rattus	Mice	Musk rats	*G. varius*
235	51	22	0

Average fleas per *M. rattus*	8·1
Number of traps set	685
All rats per 100 traps set	45·0
M. rattus per 100 traps set	34·4

The conditions observed in Bhagalpur were found to differ so remarkably from those found in the towns and villages examined in Eastern Bengal that we decided to examine another town lying between Bhagalpur and the boundaries of the province of Eastern Bengal and Assam and which had not suffered from plague. A town answering this purpose was found in Purneah.

(4) *Purneah.* (Figs. 11, 12, 13.) This town is the capital of the district of the same name. It is situated only 50 miles from Bhagalpur in the old silted up bed of the Kosi river. It had a population of 14,000 in 1901. The inhabitants are scattered over an area of about fifteen square miles and the houses are grouped into three practically distant villages. The part of the town where trapping was carried out consists of one or two main streets which are very wide (as may be seen in fig. 11) with rows of houses, chiefly shops, on each side of the road. Beyond the main streets the houses or groups of houses are sometimes so secluded by trees and widely separated as to be hardly in sight of one another. Trapping commenced here on the 10th March and was continued to the 16th. The results are summarised below.

Rats caught :

M. rattus	Mice	Musk rats	*G. varius*
22	14	54	1

Average fleas per *M. rattus*	4·9
Number of traps set	432
All rats per 100 traps set	21·1
M. rattus per 100 traps set	5·1

(5) *Raibazaar.* The next place visited was Raibazaar, a large village situated some four miles from Dacca, not far from the river, on

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fairly elevated and well-drained land. The village was selected because it was inhabited by a large number of potters who, some generations ago, had come from the town of Rajmhal near Bhagalpur in Behar. The houses in this village differed a good deal from those of an ordinary Bengali village especially in that a few of them had tiled roofs. The population of the village was about 3931. Trapping began on the 20th March and continued to the 3rd of April with the following results.

Rats caught:

M. rattus	Mice	Musk rats	*G. varius*
49	31	16	38

Average fleas per *M. rattus*	...	...	5·4
Number of traps set	...	...	541
All rats per 100 traps set	...	...	24·8
M. rattus per 100 traps set	...	...	9·1

(6) *Dibrugarh.* (Figs. 21, 22.) The town is one of the largest in Assam and is situated on the left bank of the Brahmaputra river. The population numbered 11,227 in 1901. The inhabitants are Assamese, Bengalis, Beharis, Marwaris and a large number of other races from almost every part of India, who have migrated thither to work on or cater for the extensive tea gardens in its neighbourhood. In the Marwari quarter rows of shops and grain godowns are contiguous with one another along the river bank. Recent floods have destroyed a portion of this quarter and it was here that the small outbreak of plague occurred in 1903. The results of trapping the town are as follows.

Rats caught:

M. rattus	Mice	Musk rats	*G. varius*
6	9	12	0

Average fleas per *M. rattus*	...	...	3·5
Number of traps set	...	...	423
All rats per 100 traps set	...	...	6·4
M. rattus per 100 traps set	...	...	1·4

We may conclude from the above experience that except in Bhagalpur the number of rats (*M. rattus*) caught in the towns or villages visited was never greater than eleven for every 100 traps set.

On account of the small number of rats caught (except in Bhagalpur) the flea counts on the rats are not of much value. In the latter place, however, the flea count in the beginning of March, viz. 8·1, was somewhat higher than the count made in Dacca at the same time. It is of

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interest to note also that among the fleas found on rats in Bhagalpur about 2 % belonged to the species *Ceratophyllus fasciatus*. A single specimen of a new species of flea was captured on a rat in Jatrabari village which has been named by Rothschild *Acropsylla episema* (gen. et sp. nov.)[1].

	Average *M. rattus* per 100 traps	Average fleas per rat
Dacca	7	4·2
Chittagong	10	3·8
Jatrabari	2 4	2·3 0·3
Bhagalpur	34	8·1
Purneah	5	4·9
Raibazaar	9	5·4
Dibrugarh	1	3·5

G. Deductions to be drawn from the above Data.

(1) *The freedom of Eastern Bengal from plague is due mainly to the scarcity of rats in the houses of the people.*

The number of rats (*M. rattus*) caught in every 100 traps set in Dacca town and in the other towns and villages of Eastern Bengal and Assam visited by the Commission varied from about two in Jatrabari and Dibrugarh to about ten in Dacca and Chittagong. In none of these places, with the exception of Dibrugarh, has bubonic plague ever occurred in epidemic form. Even in Dibrugarh the epidemic, which was of the mildest type, did not last more than a few weeks. In Bhagalpur, which is the nearest plague-infected town to Eastern Bengal (Calcutta excepted), as many as 34 *M. rattus* were caught in every 100 traps set.

In other places in India visited by the Commission *M. rattus* has been caught much more freely. All these places are liable to epidemics of plague.

	Rats per 100 traps
Belgaum (x. p. 474)	13—37; average 23
Poona (x. p. 520)	16—43; ,, 28
Dhand (vii. p. 912)	— ; ,, 26
Kasel (vii. p. 913)	— ; ,, 54
Parel (vii. p. 847)	9—65; ,, 24
Worli (vii. p. 868)	10—70; ,, 22

Fleas on the other hand appear to be about as abundant in the province as elsewhere, varying from about two to eight per rat.

[1] N. C. Rothschild, *Novitates Zoologicae*, Vol. xviii. (1911), p. 118.

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Comparable figures from plague-infected places previously examined are as follows.

	Fleas per rat
Belgaum (x. p. 461)	3—19; average 10
Bombay (VIII. p. 297)	2—5 ; ,, 4
Dhand (VII. p. 915)	2—12; ,, 9
Kasel (VII. p. 915)	2—13; ,, 8
Poona (x. p. 525)	2—9 ; ,, 6

In spite of the defects and fallacies of our method of estimating the rat infestation of a place by the number of rats caught in every 100 traps set (fallacies which we have recognised and striven to avoid), we think, in view of the remarkable differences in the figures obtained in this way in Eastern Bengal and Assam and in other parts of India, that we are justified in stating that the main reason for the freedom of Eastern Bengal and Assam from plague depends on the scarcity of rats in the houses of its inhabitants.

(2) *The physical features of the country assist in keeping the province free from plague.*

We have seen that the physical features of the province protect it to some extent from the importation of infection. The nearest plague-infected centres are separated from the province by intervening plague-free areas and the Ganges river. And, although communication by rail with infected centres exists, it is interrupted at places by the great rivers. Roads are few and of an indifferent character while travelling by the rivers and canals although easy is yet slow. Extensive floods during many months of the year still further interrupt free communication between village and village and even between individual homesteads. The villages consist of scattered hamlets built on raised mounds of earth which are spread over an extensive area (see figs. 3 and 4) so that even if plague broke out among the rats in a village infection could only be spread through human agency, the rats in one part of a village being cut off from the rats of another part by long stretches of low-lying ground which is often flooded. The abundant rainfall, ample water supply and fertile soil have prevented famines, so that the people are rich in comparison with those of other parts of India. Sufficient crops are produced for the immediate needs of the inhabitants, so that any trade in grain is in an outward direction while the only imports are of such a kind as are little likely to carry plague infection in the form of infected rats or rat fleas.

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H. The Causes of the Comparative Scarcity of Rats in the Houses of Eastern Bengal.

We have shown that the freedom of Eastern Bengal and Assam from plague may be attributed chiefly to the small number of rats found in the houses. We must now consider shortly some of the reasons which have brought about this desirable state of affairs.

(1) *The habits of the people.*

We have stated that, owing to the physical features of the country, famines have been entirely absent from the land. The people are therefore richer than those in many other parts of India, and they have accordingly adopted many European habits and customs. Articles of European pattern are frequently to be found in their dress and the use of beds and chairs is common even among the poorer classes. But a striking characteristic of the Bengali is his neatness and tidiness especially compared with the inhabitants of other parts of India. This habit pertains not only to his person but extends to his house and its surroundings. Even within the simple matting huts of the villages one can see pillows, bottles, lamps, utensils and other articles of daily use neatly hung up in a symmetrical manner on the walls. After sweeping and cleaning out the house in the morning nothing unnecessary appears to be left on the floor. How far this habit is due to the experience derived from the floods which occasionally penetrate the houses we are unable to say; it remains, however, a striking feature almost peculiar to the inhabitants of this part of India. But order extends beyond the Bengali home. Around the houses one seldom sees rubbish littered about as is so common in towns and villages in other parts of India. The ground about the house is usually swept, and materials which are of value are generally neatly stacked in piles or bundles. Among many defects in Indian character there is none greater than that which permits him to be satisfied with things that are temporary, tawdry, makeshift or "kutcha." What is good enough for to-day is sufficient for him, he has no consideration for the future. For this reason when an Indian builds a house he generally does so in a haphazard and makeshift manner. When the house is built very little thought is given to its repair, but when this becomes absolutely necessary it is effected by the first materials which come to hand. The Indian seldom

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takes any pride in the beauty and neatness of his home. As a result of these slovenly habits the majority of the houses in an Indian village are in a condition representing every stage of dilapidation and ruin.

Fortunately the Bengali suffers less from this defect than the average Indian. He does sometimes take a pride in his home and it is said that one of the greatest assets in the estate of a man of means is a "pucca" family dwelling which can be handed down from father to son. A house is thus built which is expected to last for at least one generation. Where, as in the towns, lime and cement can readily be obtained and wealth is greater a "pucca" house is constructed while, even in the villages, where these materials are scarce, a house is made of corrugated iron imported from Calcutta or is built of neatly spliced bamboo matting with a roof of thatch which is kept in good repair. Nothing can bring home more forcibly the differences between the habits of the Bengali which have been mentioned above and the ordinary Indian villager than a glance at the accompanying photographs. Figures 5 and 6 were taken in a village near Dacca. Figures 13 and 14 were taken in Purneah and Puradah in Bengal. The tidiness, order, symmetry in these villages are in marked contrast to the conditions shown in figures 15 to 18, which were taken in plague-infected Bhagalpur. Here disorder and dilapidation seem to court the presence of the plague.

(2) *The structure of their houses.*

While the habits of the people may to some extent account for the absence of rats from their homes, yet the construction of the houses leads to the same end. As a matter of fact, whether intuitively or otherwise, the Bengali seems to have adopted a method of house construction which succeeds in practically eliminating rats from his house, be the house a pucca or a kutcha one.

"Pucca" houses, being built of stone or brick or mortar, often with concrete roof, from the very nature of the materials used in their construction afford little harbourage for rats. These animals may however effect an entrance from outside either through the floor or by the door. Many Bengali houses however have been so constructed as to prevent rats gaining an entrance in these ways, being built on raised plinths of special construction. These plinths are erected with a brick or masonry facing, along the upper border of which runs a bead of varying depth which projects for from four to six inches or more over the base. The floor is made of a layer of concrete faced with

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hard polished cement, below which is a layer a foot or more deep of dry sand. These devices are said to have been adopted for a number of reasons: (1) to keep the floors dry, a very necessary precaution in a country so subject to floods; (2) to prevent white ants from entering the houses; (3) to prevent the access of rats which cannot make their burrows in the sand or climb over the overhanging plinth. Of course not all the native houses are built in this way but nearly all the new government buildings at Dacca are constructed on this plan.

The "kutcha" houses, too, afford little shelter for rats. For, being constructed of thin bamboo matting or wattle covered with a thin layer of mud, the walls afford no shelter for these rodents. The roofs too, being made of corrugated iron, split bamboo, or thin grass thatch, fail to shelter these animals. Perhaps one of the most noteworthy features of the buildings in this province is the almost complete absence of tiled roofs which are so commonly seen in many other parts of India. Even the houses which are built of mud here differ from those seen in other places. The walls are as thin as possible consistent with stability, being often only ten inches to one foot in thickness. Under these circumstances the entire weight of the corrugated iron or thatch roof has to be taken off the walls by erecting it on four or more bamboo poles. This roof, as will be seen from the photographs 9 and 10, is an entirely separate structure from the mud building; projecting beyond the mud walls, it acts as a shelter protecting them against the sun and rain. The real roof of the building or ceiling of the rooms is made of a thin layer of bamboo battens plastered over with mud. It is said that the chief reason for constructing the houses in this way is to prevent rats burrowing in the walls which are so thin that they fail to find sufficient shelter or security within them. The walls of mud houses in other parts of India are made of such a thickness that rats find ample room to make their burrows within them. Photographs 17 and 18 taken in Bhagalpur demonstrate this fact. Living in this situation the rats not only find a warm and sheltered home but are in close proximity to their food supply which is always abundantly found within and around the untidy Indian dwelling. It will thus be seen that in Eastern Bengal and Assam it is difficult to decide whether "pucca" or "kutcha" houses afford the more shelter for rats; it is evident, at all events, that the structure of the houses here in conjunction with the habits of the people conduce to the comparative scarcity of rats in their dwellings and are the main reasons for the freedom of the province from plague.

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Some examples of the various types of buildings found in different parts of Eastern Bengal and Assam are shown in Plates V—XIV. Photograph 7 shows to what extent pucca houses have been built in Dacca town. Photograph 8 shows a group of kutcha buildings in the same place. Photographs 19 and 20 are views taken in Chittagong which illustrate the structural peculiarities of the houses within the town and the suburbs. Photographs 21 and 22 depict the type of houses seen in Dibrugarh in Assam.

I. General Conclusions.

(1) The province of Eastern Bengal and Assam has suffered very little from bubonic plague; a few epidemics only of pneumonic plague have occurred.

(2) The physical features of the country protect it to some extent from the importation of infection and would tend to limit the opportunities for spreading the disease if it once broke out.

(3) The freedom of the province from plague can chiefly be attributed to the scarcity of rats in the houses as compared with other parts of India.

(4) *M. rattus* is comparatively rare in Bengali houses because of the habits of these people in respect to their greater regard for neatness and tidiness both in and around their dwellings which diminishes the food supply of the rodents.

(5) The structure and design of the Bengali home, whether it be of the solid masonry type on the one hand or of the flimsy matting or grass type on the other, afford little shelter for rats.

(6) The presence of natural enemies of *M. rattus* such as the musk rat may assist in maintaining a low rat infestation of the houses.

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Fig. 1.

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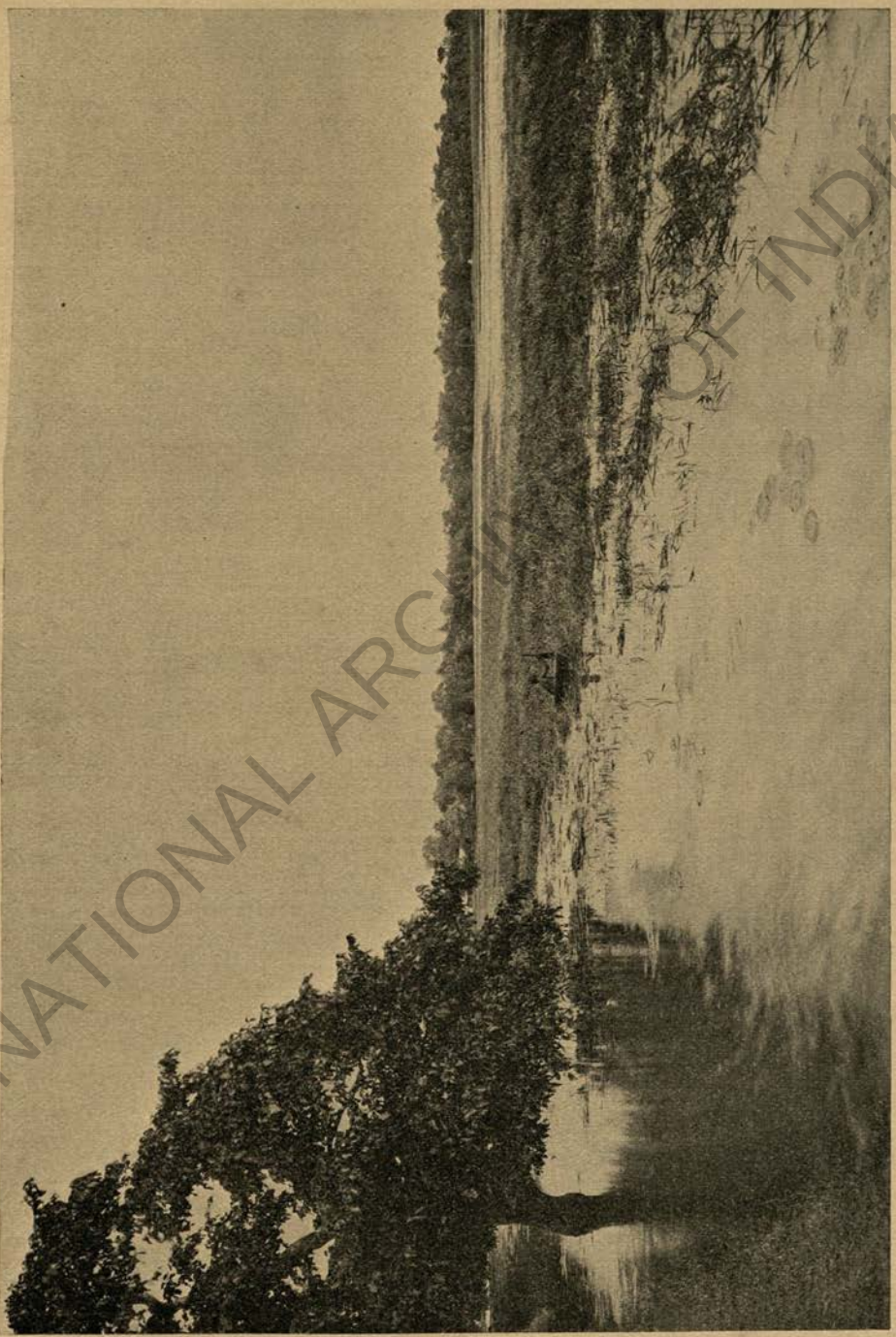

Fig. 2.

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Fig. 3. Jatrabari village, showing scattered hamlets.

Fig. 4. Jatrabari village: a hamlet.

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Fig. 5. Village near Dacca.

Fig. 6. Village near Dacca.

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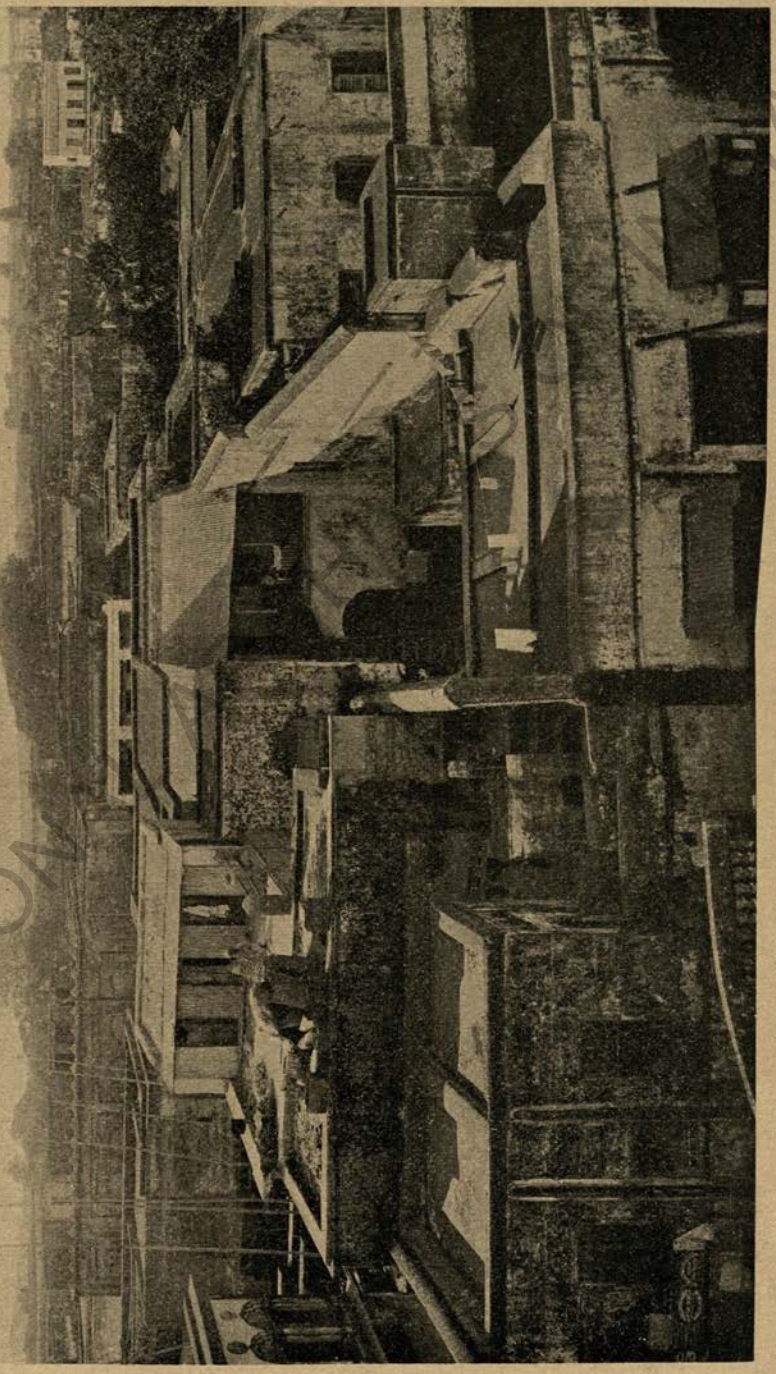

Fig. 7. Dacca: "pucca" houses.

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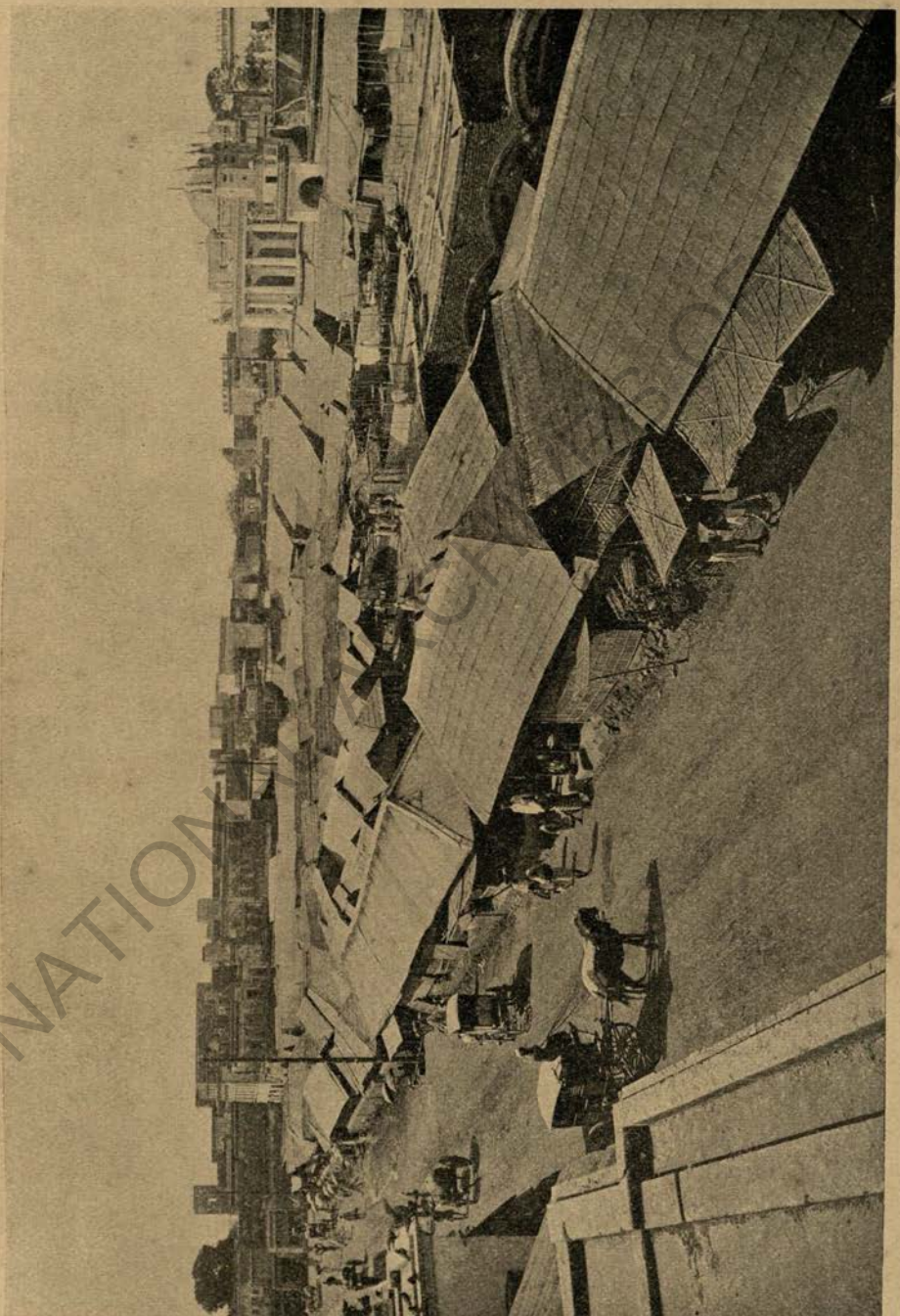

Fig. 8. Dacca: "kutcha" houses.

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Fig. 9. Showing method of supporting roof: Dacca.

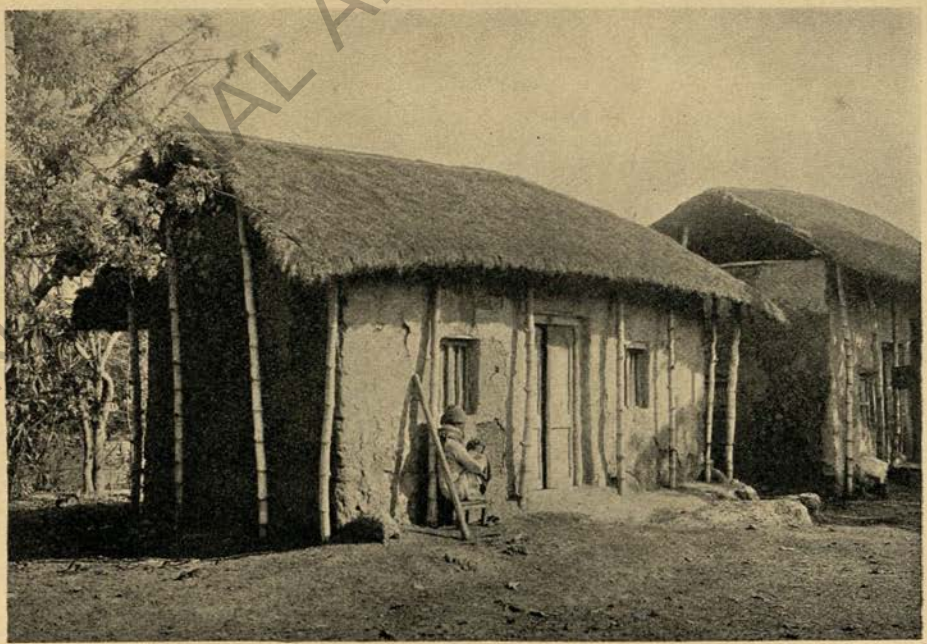

Fig. 10. Showing method of supporting roof: Dacca.

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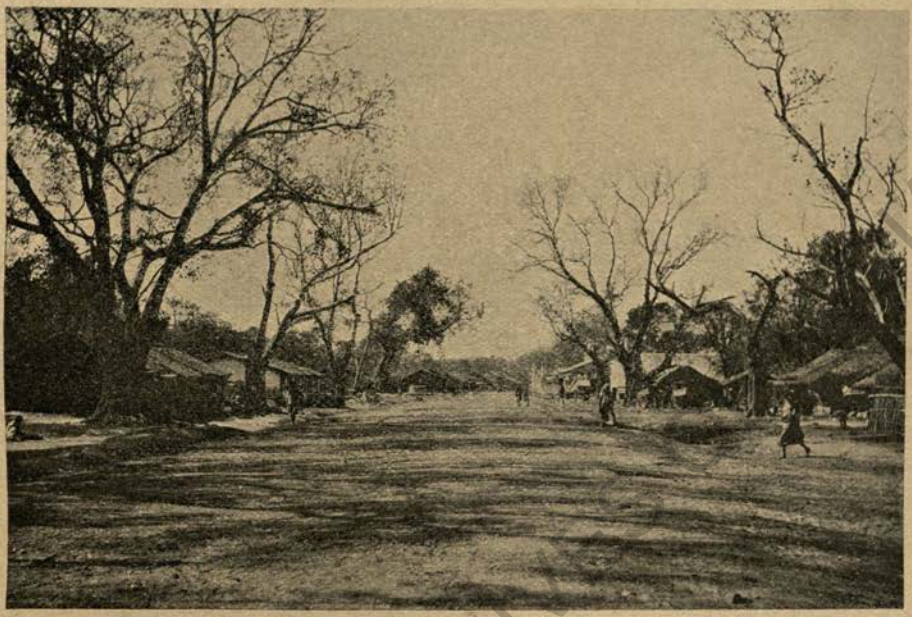

Fig. 11. Purneah: free from plague.

Fig. 12. Purneah: free from plague.

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Fig. 13. Purneah: free from plague.

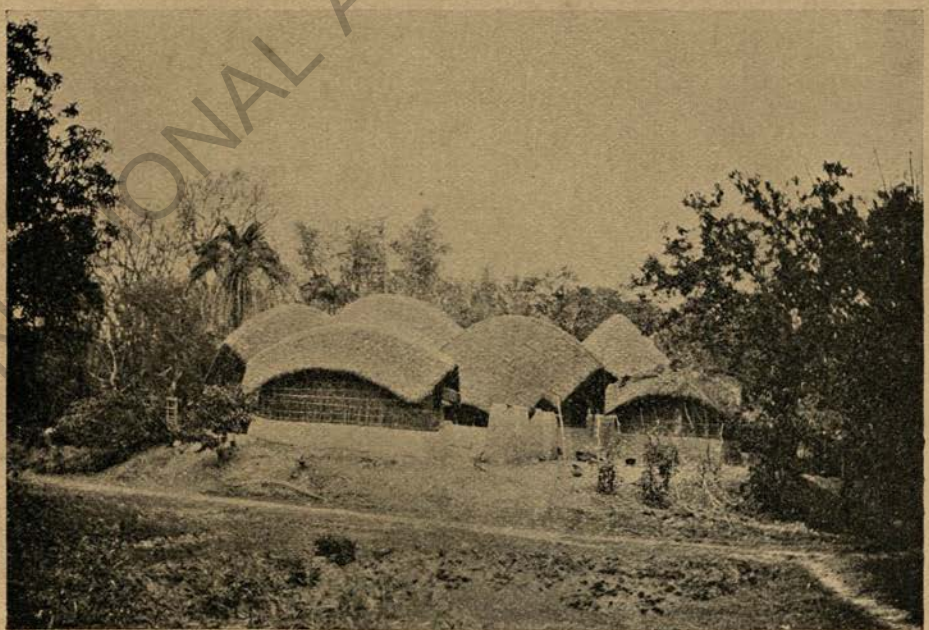

Fig. 14. Puradah, a village in Lower Bengal: free from plague.

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Fig. 15. Bhagalpur: plague infected.

Fig. 16. Bhagalpur.

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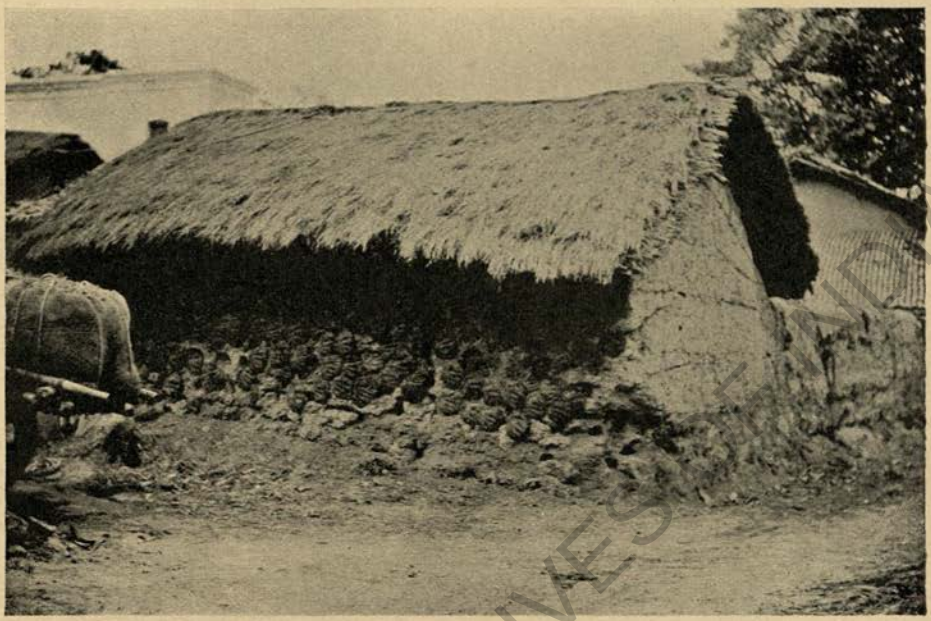

Fig. 17. Bhagalpur: mud walls and rat holes.

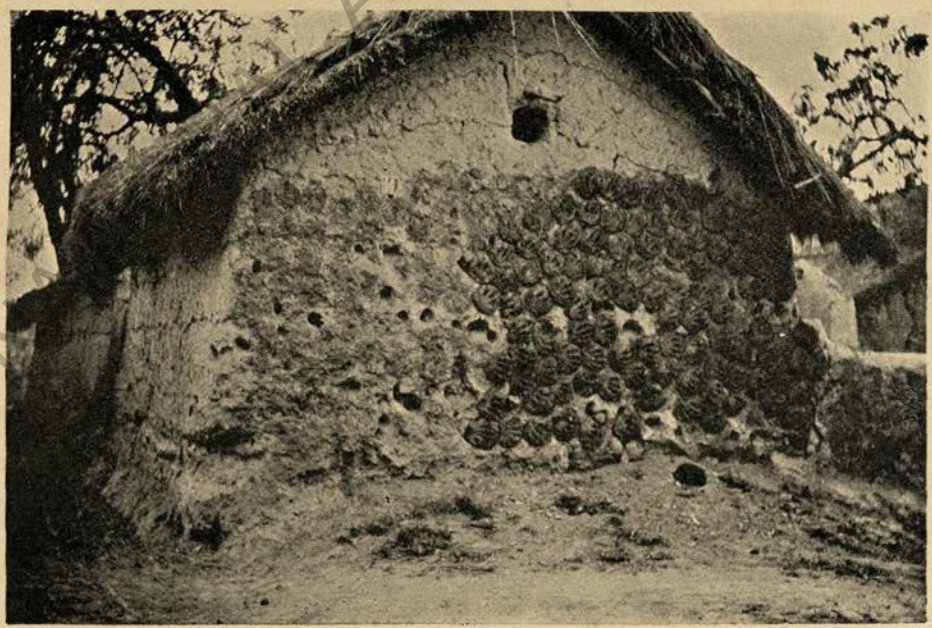

Fig. 18. Bhagalpur: mud walls and rat holes.

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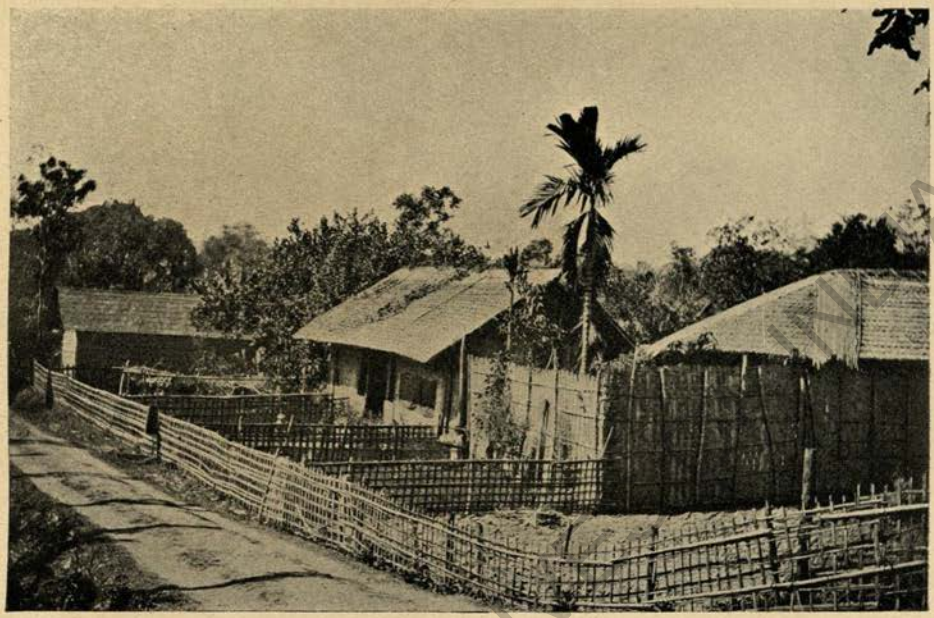

Fig. 19. Chittagong.

Fig. 20. Chittagong.

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Fig. 21. Dibrugarh.

Fig. 22. Dibrugarh.

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XLVII. OBSERVATIONS ON THE BREEDING OF *MUS RATTUS* IN CAPTIVITY.

With 1 Chart in Text.

THE present series of experiments on the breeding of *Mus rattus* from wild stock was undertaken in order to obtain reliable data as to the fecundity and breeding habits of this rat. The information was necessary for a proper appreciation of the possibilities of the re-establishment of a rat population after epizootic plague or after destructive measures. The young rats obtained were also required for certain immunity experiments and it was hoped also to obtain some general information as to the rate of growth of rats and other biological facts.

Most of the data available as to the fecundity of rats are based on observations made on *Mus decumanus* (*norvegicus*), but as *Mus rattus* is the one most concerned with the spread of plague in India our breeding experiments were done with this species only.

Attempts to breed from wild *Mus rattus* in cages are generally unsuccessful. These rats will rarely breed at all in cages, and if a litter happens to be born the young ones are usually eaten by their parents within a few days of birth. Knowing this difficulty of breeding in cages, special arrangements were made to breed under conditions in which the natural habitat of *Mus rattus* was more or less satisfactorily reproduced. Six special godowns were built. These were small cabins about six feet square by seven feet high, cement lined and rat and flea proof. In the floor of each godown four small cement lined chambers, with removable lids flush with the floor, were sunk. An artificial burrow consisting of an iron pipe sunk in the floor led into each of these nests. These pipes were about two feet long and of sufficient diameter to admit one rat at a time. A bend in the pipe kept the nest into which it led almost completely dark. Rats let loose in the godowns could run about the floors or climb on the metal work of the double doors or retire down the pipes to their nests, and, in general, have very much the same freedom of movement as under natural wild conditions.

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As will be seen, breeding was successfully accomplished in these godowns and a considerable number of litters were reared.

On the 20th July, 1909, the following pairs of rats were put into the godowns:

Godown No. 1	...	...	One pair.
,, ,, 2	...	...	Two pairs.
,, ,, 3	...	...	Four pairs.
,, ,, 4	...	...	Eight pairs.
,, ,, 5	...	...	Sixteen pairs.
,, ,, 6	...	...	One pair.

The result of this arrangement was as follows:

Godown No. 5: 16 *pairs.* Up to 2nd Sept. 1909 eight males and eight females died, probably from fighting, many of them being eaten. The remaining eight pairs were then killed and examined but none of the females were found pregnant. Four fresh males and twelve females were then put in and left until 25th Sept. when they were removed to make room for a successfully breeding pair from godown No. 6. None of the twelve females were pregnant.

Godown No. 4: 8 *pairs.* Four males died up to 20th Sept. The number was then reduced to one pair. None of the females removed were pregnant.

Godown No. 3: 4 *pairs.* A litter of six was born on 13th Sept. but was eaten on 17th Sept. The number was reduced to one pair on 20th Sept. None of the females were pregnant.

Godown No. 2: 2 *pairs.* One male died on 13th Sept. and one female on 17th Sept. One pair was thus left which commenced breeding, a litter being born on 15th Oct.

Godown No. 6: 1 *pair.* A litter of five was born on 2nd Sept. On 25th Sept. the parents were removed to godown No. 5.

Godown No. 1: 1 *pair.* The female killed three successive males on 8th Aug., 18th Aug., and 11th Nov. She was removed on 11th Nov. and kept till 6th Dec. and then killed and found not pregnant.

It will be seen that the first litter to be successfully reared was in godown No. 6 with only one pair of rats. The second litter successfully reared was in godown No. 2 which originally contained two pairs but in which breeding did not take place until after the death of one pair. The only litter born in the godowns containing more than one pair was in godown No. 3 and the young ones were eaten within four days of birth. The failure to breed of the pair in godown No. 1 was probably due to the female being old and unsuitable.

It thus appears that the most satisfactory arrangement to obtain successful breeding is with only one pair in each godown. From 20th Sept. 1909 onwards this arrangement was adopted and numerous litters were reared successfully.

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Breeding History of Successful Pairs from 20th Sept. 1909 onwards.

Pair in Godown No. 2.

The following litters were born:

15th Oct. 1909	3 born	Male removed.
17th Nov. ,,		Male returned to godown.
11th Dec. ,,	6 born	Male not removed.
13th Dec. ,,		Two young ones eaten. Male removed.
10th Jan. 1910		Male returned to godown.
13th Feb. ,,	6 born	Some showed tooth marks. Male removed.
14th Feb. ,,		Female has eaten one.
21st Feb. ,,		One more eaten by female, head only left.
1st Mar. ,,		Male returned to godown.
4th Mar. ,,	6 born	Male not removed.
5th May ,,	5 born	Male removed.
2nd June ,,		Male returned to godown.
10th Aug. ,,	8 born	All eaten on the same day.

Pair in Godown No. 3.

The following litters were born:

9th Nov. 1909	6 born	Male removed.
27th Nov. ,,		Male returned to godown.
18th Jan. 1910	8 born	Male not removed.
31st Mar. ,,	7 born	Male removed
20th Apr. ,,		Male returned to godown.
25th May ,,	5 born	Male not removed.
10th Aug. ,,	7 born	,, ,,
8th Oct. ,,	6 born	,, ,,
30th Dec. ,,	4 born	,, ,,

Pair in Godown No. 4.

Put in on 6th Dec. 1909. The following litters were born:

7th Feb. 1910	6 born	Male removed.
26th Feb. ,,		Male returned to godown.
20th April ,,	5 born	Male removed.
25th April ,,		Two eaten by female.
26th April ,,		Three eaten by female. Male returned to godown.
23rd June ,,	6 born	Male removed.
25th June ,,		Whole litters eaten by female.

Pair killed off on 29th Sept. 1910 and one fresh male and two females put in.

10th Feb. 1911	7 born	Male not removed.

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Pair in Godown No. 5.

The following litters were born:

2nd Sept. 1909	5 born	This litter was born in godown No. 6 and was transferred to this godown on 25th Sept. 1909. Male not removed.
6th Nov. ,,	1 found	This rat was apparently several days old and was possibly one of a litter several of which may have been eaten. Male not removed.
20th Nov. ,,	5 born	Male not removed.
3rd Jan. 1910	5 born	,, ,,
21st Feb. ,,	3 born	,, ,,
2nd May ,,	5 born	,, ,,
30th June ,,	5 born	,, ,,

Pair killed on 29th Sept. 1910.

Pairs in Godowns Nos. 1 *and* 6.

No litters were obtained.

Second Series of Godowns.

A second series of breeding godown was started on 19th Oct. 1910, one male being placed in each godown with one or more females. A few litters have been obtained.

Godown No. 7.

One male and three females.

16th Dec. 1910	litter born	Number unknown as all were eaten up with the exception of parts of heads and tails.
8th Mar. 1911	6 born	Two females and the male removed.

Godown No. 8.

One male and one female.

17th Jan. 1911	4 born	Male removed.

Godown No. 9.

One male and two females.

3rd Mar. 1910	5 born.

Godown No. 10.

One male and two females.

No breeding.

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Godown No. 11.

One male and three females.

7th Feb. 1911	5 born	Mother and young removed.
12th Feb. ,,		All eaten by mother.
12th Feb. ,,	7 born	From second female.

Godown No. 12.

One male and three females.

No breeding.

Breeding of a second generation.

A male and female from separate litters born in the godowns were placed in a large specially constructed cage which contained a box lined with straw in imitation of the breeding chambers of the godowns.

The male of this pair was from the litter born in godown No. 2 on 15th Oct. 1909 and the female from the litter born in godown No. 5 on 2nd Sept. 1909.

The following litters were born:

19th Feb. 1910	7 born	Male removed.
2nd Mar. ,,		Six eaten by female, remaining one removed and male returned to cage.
30th May ,,	6 born	Male not removed.
17th Oct. ,,	6 born	,, ,,
2nd Jan. 1911	6 born	Male removed.
11th Jan. ,,		Four eaten by female, remaining two dead. Male returned.
13th Feb. ,,	8 born	

The results obtained in the most successful of these godowns supply us with figures from which we can get a fair idea of the fecundity of *Mus rattus*. Of course the temporary removal of the males in some of the godowns, after the birth of a litter, may have prevented the females again becoming pregnant as soon as might otherwise have happened, and the frequency of litters was perhaps diminished in this way. The artificial conditions may also have diminished the natural rapidity of breeding.

A short summary of the litters obtained from pairs giving three or more litters will provide the figures on which to base a calculation of fecundity.

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Pair in Godown No. 2.

Date	Number born
15th Oct. 1909	3
11th Dec. ,,	6
13th Feb. 1910	6
4th Apr. ,,	6
5th May ,,	5
10th Aug. ,,	8

Total 34 born in 9 months and 26 days.

Pair in Godown No. 3.

9th Nov. 1909	6
10th Jan. 1910	8
31st Mar. ,,	7
23rd May ,,	5
10th Aug. ,,	7
8th Oct. ,,	6
30th Dec. ,,	4

Total 43 born in 13 months and 20 days.

Pair in Godown No. 4.

Date	Number born
7th Feb. 1910	6
20th Apr. ,,	5
23rd June ,,	6

Total 17 born in 4 months and 13 days.

Pair in Godown No. 5.

20th July 1909	5
6th Nov. ,,	1
20th Nov. ,,	5
3rd Jan. 1910	5
21st Feb. ,,	3
2nd May ,,	5
30th June ,,	5

Total 29 born in 11 months and 10 days.

Pair in Breeding Cage No. 1.

19th Feb. 1910	7
30th May ,,	6
17th Oct. ,,	6
2nd Jan. 1911	6
13th Feb. ,,	8

Total 33 born in 11 months and 22 days.

Rate of increase of rats.

Working from these figures showing the number of young born in a given period, we can calculate roughly the number of young which an actively breeding pair will produce in a year. Allowing three weeks for the first incubation, the following approximate figures are obtained for four successful pairs:

Pair in Godown	No. 2	39 in 12 months.
,, ,,	No. 3	36 ,,
,, ,,	No. 4	44 ,,
,, ,,	No. 5	29 ,,
,, Cage	No. 1	32 ,,

This gives an average of 36 in twelve months or a litter of six (the commonest number) every two months.

The following figures show the number of rats in our litters:

Litters of one	... ...	doubtful
,, two	... ...	nil
,, three	... ...	2
,, four	... ...	2
,, five	... ...	10
,, six	... ...	12
,, seven	... ...	5
,, eight	...	3

Average litter 5·7. Commonest litter 6.

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These figures may further be applied to the calculation of the total progeny of a pair in a given period allowing for the coming to maturity and the commencement of breeding of successive generations of young rats. We assume that each litter consists of six, and we take it for granted that a litter can be produced when the rats reach the age of five months. The age of five months as the starting point for breeding is based on the pair, details of whose breeding of a second generation have already been given. The male of this pair was four months old at the time of the birth of the first litter, and the female 5½ months. The rats however were, to external appearances, sexually mature by the end of the third month, and under natural conditions would probably breed at an earlier period than five months. In the table which Boelter gives for *decumanus* the first litter is taken as being born when the parents are 3½ months old.

Our table also assumes that at least as many females are born as males. Our figures show more females than males to have been born, and also that polygamy occurs. It will thus be seen that the figures taken err, if anything, on the side of under estimation. The calculations show that a single pair may produce 198 young in 12 months and 858 in 16 months. This does not allow for destruction of young by their parents or by natural means.

Intervals between the births of litters.

When the male had continued access to the female, the following intervals occurred between two litters:

29 days, 31 days, 44 days, 47 days, 49 days, 70 days, 72 days, 80 days, 82 days.

Average interval—56 days.

When the males were removed for varying periods the following intervals elapsed between re-introduction of the male and a birth:

24 days, 33 days, 42 days, 50 days, 53 days, 58 days, 78 days.

Average interval—48 days.

Out of 55 born in the godowns

24 were males (43·6 %)
31 were females (56·4 %)

For comparison we give the percentage of the sexes of wild rats caught in Bombay (calculated on over 30,000):

Males 51 %
Females 49 %

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Polygamy.

The results in godown No. 11 show definitely the occurrence of polygamy. In this godown there was one male with three females. Two of the females gave birth to a litter within five days of each other, the one litter consisting of five and the other of seven.

Eating of young rats by the parents.

Out of 35 litters, ten were eaten by the parent rats. Of these ten litters, six were eaten by the female alone and the remaining four litters were eaten by either the male or female. The extent to which this destruction of young goes on under wild conditions is unknown. In our godowns destruction may have been unnaturally large owing to the handling of the young rats for the purpose of weighing and measuring and to the absence of flesh diet. Throughout the whole period of the experiments the only food in the godowns was parched rice or radish. No flesh was given at any time. The rats remained healthy and fat on this diet. There may, however, have been a craving for flesh which led to the eating of the young, and it has frequently been observed that rats in cages will eagerly devour raw flesh or other rats when they will entirely refuse to touch vegetable food. Handling could not have been a factor in many cases for some litters were eaten which had never been touched. It is possible that the same destruction goes on in nature even when some flesh food is available, but we can have no accurate guide as to its extent.

Rate of Growth of Young Rats.

Records of weights, head and body lengths and tail lengths were kept for several litters for varying periods up to four months after birth. The tails were sometimes broken off in handling or bitten off by the rats themselves when fighting, and for this and other reasons the measurements could not be continued as long as might have been desired. The main facts, however, as to rate of growth are shown in the following tables which give the averages for each litter.

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TABLE I.

Average weights and measurements of young rats.

Litter of six born 9th Nov. 1909.

Number of days after birth	Weight (in grammes)	Length of head and body (in millimetres)	Length of tail (in millimetres)
At birth	4·6	—	—
6	10·8	59	41
13	20·0	80	83
16	22·8	—	—
20	23·0	96	115
27	27·5	104	132
34	38·3	110	149
41	40·5	117	151
48	48·5	134	160
55	57·0	137	164
62	65·0	140	170
69	70·3	143	173
76	76·0	146	177
83	77·5	—	—
90	83·3	—	—
97	84·0	—	—
104	86·6	—	—
111	92·1	—	—
125	98·6	166	188
139	100·0	167	188

TABLE II.

Average weights and measurements of young rats.

Litter of five born 20th Nov. 1909.

Number of days after birth	Weight (in grammes)	Length of head and body (in millimetres)	Length of tail (in millimetres)
At birth	4·2	—	—
1	5·0	49	22
2	5·8	—	—
3	6·6	—	—
5	9·0	—	—
9	14·0	70	56
12	17·4	—	—
13	—	83	76
16	21·6	87	95
23	25·8	98	118
30	31·0	108	133
37	40·0	122	143
44	50·0	126	153
51	58·0	129	165
58	66·6	139	169
65	71·8	143	178
72	82·0	144	186
79	89·0	149	190
86	96·6	155	192
93	98·6	—	—

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TABLE III.

Average weights and measurements of young rats.

Litter of six born 11th Dec. 1909.

Number of days after birth	Weight (in grammes)	Length of head and body (in millimetres)	Length of tail (in millimetres)
At birth	4·1	41	20
2	6·0	48	22
4	7·7	57	30
6	10·7	60	35
9	16·0	69	48
16	27·7	94	92
23	40·0	118	123
30	54·0	126	155
37	59·2	130	165
44	63·5	140	170
51	74·0	143	175
58	82·2	146	180
81	110·6	158	199

TABLE IV.

Average weights and measurements of young rats.

Litter of five born 3rd Jan. 1910.

Number of days after birth	Weight (in grammes)	Length of head and body (in millimetres)	Length of tail (in millimetres)
At birth	4·2	44	18
1	5·0	—	—
2	5·8	49	24
4	8·0	62	33
5	9·2	—	—
7	11·4	67	44
9	13·8	72	53
11	16·4	77	64
14	19·4	86	85
17	22·0	91	103
21	27·0	101	122
23	28·0	103	127
28	28·0	104	133
35	36·0	108	143
42	42·0	117	150
49	49·7	128	157
56	60·5	133	166
63	65·8	136	174
70	74·0	145	183
77	82·2	147	185
105	115·0	165	192

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TABLE V.

Average weights and measurements of young rats.

Litter of six born 18th Jan. 1910.

Number of days after birth	Weight (in grammes)	Length of head and body (in millimetres)	Length of tail (in millimetres)
At birth	4·1	45	22
1	4·5	—	—
2	5·2	52	26
3	6·1	—	—
6	9·3	63	40
8	11·5	68	47
11	14·6	77	65
13	16·6	87	87
20	24·1	94	113
27	29·0	104	139
34	33·0	115	147
41	38·7	118	149
48	47·2	125	158
55	49·8	131	165
62	57·0	134	168
69	63·0	142	175
76	64·0	143	178
83	67·5	144	178
90	68·2	147	180

TABLE VI.

Average weights and measurements of young rats.

Litter born on 7th Feb. 1910.

Number of days after birth	Weight (in grammes)	Length of head and body (in millimetres)	Length of tail (in millimetres)
At birth	4·7	44	20
2	5·5	51	23
4	7·0	57	29
7	9·1	65	40
9	10·6	68	47
11	12·0	74	58
14	14·1	80	68
19	16·0	79	99
28	22·2	98	103
35	27·0	108	117
42	32·6	113	128
49	39·0	123	134
56	51·6	128	147
63	58·1	138	160
77	76·0	141	162

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TABLE VII.

Average weights and measurements of young rats.

Litter of three born 21st Feb. 1910.

Number of days after birth	Weight (in grammes)	Length of head and body (in millimetres)	Length of tail (in millimetres)
At birth	4·0	48	22
2	6·0	53	27
5	10·0	62	45
7	13·3	71	53
11	20·0	82	78
14	24·0	90	96
Removed from mother and put in cage No. 17.			
21	22·0	101	109
28	27·3	105	137
35	32·0	114	145
42	40·0	117	155
49	43·3	125	164
56	51·0	128	165
66	55·3	134	168

TABLE VIII.

Table of average weights calculated from seven litters.

Age	Weight
At birth	4·3 grammes
1 week	11·3 ,,
2 ,,	18·3 ,,
3 ,,	24·7 ,,
4 ,,	30·4 ,,
5 ,,	37·7 ,,
6 ,,	43·4 ,,
7 ,,	50·9 ,,
8 ,,	59·4 ,,
9 ,,	65·5 ,,
10 ,,	73·6 ,,
11 ,,	80·4 ,,
12 ,,	86·0 ,,

Weight. It will be seen that the weight at birth varied from 4 grammes to 4·7 grammes, the average of seven litters being 4·3 grammes. The rate of increase of weight was approximately 1 gramme per diem up to the end of three months, beyond which period our figures are scanty. This regular increase is shown in all our tables, Table II and Table IV conforming very closely to the average growth of 1 gramme

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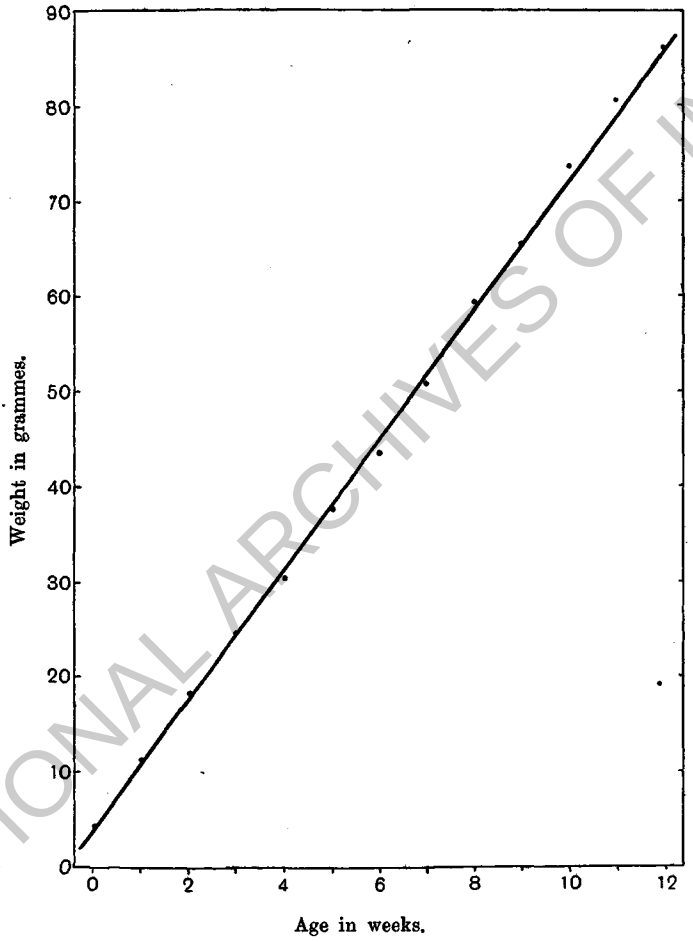
per diem. Table III considerably exceeds this while the others are slightly under it. The weights in Tables I to VII have been used to make a composite table of weights, Table VIII, in which the average weekly growth has been calculated.



The weight of a rat in grammes, minus 4 grammes for weight at birth, would according to our observations give an approximate idea of the age of the animal in days, up to a weight of about 100 grammes. This calculation would not hold for many of our rats. For example, we have one rat weighing 82·2 grammes at 58 days, while another weighs 83

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grammes at 90 days. Again, one rat weighed 100 grammes at 139 days and another 138 grammes at 123 days. On an average the calculation would however probably be a fair approximation.

Observations on many thousands of wild rats show that the majority fall into the weight groups between 100 grammes and 110 grammes. The ordinary adult rat picked at random for experimental purposes is usually of this weight and we may take it that a *rattus* of this weight may be called "grown up." *Mus rattus* therefore probably becomes "grown up" sometime in the fourth month.

Some of the tables show a diminution of the rate of growth towards the end of the third week. This is probably due to the cessation of suckling and the change to a vegetable diet. Table VII shows the result of removing the young from their mother too soon. The young rats were removed at the 14th day, and instead of gaining weight during the following week they lost an average of 2 grammes each. They continued to grow in length during this week but became very thin. They eventually became accustomed to the new diet and picked up weight. Suckling probably goes on well up to the end of the third week.

The young rats usually have their eyes open between the 11th and 15th days, the 14th being the commonest day.

Tame white rats[1] grow a good deal faster than these *rattus*, possibly because they may ultimately become larger. According to Donaldson's figures[2], starting with a weight of about 5 grammes at birth they weigh 15 after a fortnight and 30 when a month old; at 7, 10 and 12 weeks the average weights are 57, 103 and 135 grammes respectively.

Length. At birth the head and body length varies from 41 to 48 millimetres and the tail length from 18 to 22 millimetres. The tail is thus only half the length of the head and body at birth. The growth of the tail is at first very rapid and it becomes equal in length to the head and body within 12 to 16 days. At the end of the first month the tail has attained its proper length in proportion to the head and body of 25 % more, and afterwards maintains this proportion.

[1] According to Hatai (*Biological Bulletin*, Vol. XII. (1907), p. 266) these are *Mus norvegicus* (*decumanus*).

[2] *Boas Memorial Volume*, 1906, p. 5. For *Mus decumanus* see H. H. Donaldson and S. Hatai, *Journ. Comp. Neurology*, Vol. XXI. (1911), p. 417.

NATIONAL ARCHIVES OF INDIA

SEVENTH REPORT ON PLAGUE INVESTIGATIONS IN INDIA

NATIONAL ARCHIVES OF INDIA

CAMBRIDGE UNIVERSITY PRESS
C. F. CLAY, MANAGER
London: FETTER LANE, E.C.

Edinburgh: 100, PRINCES STREET
London: H. K. LEWIS, 136, GOWER STREET, W.C.
Berlin: A. ASHER AND CO.
Leipzig: F. A. BROCKHAUS
New York: G. P. PUTNAM'S SONS
Bombay and Calcutta: MACMILLAN AND CO., LTD.

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NATIONAL ARCHIVES OF INDIA

THE

JOURNAL OF HYGIENE

PLAGUE SUPPLEMENT II

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ISSUED BY

THE ADVISORY COMMITTEE

APPOINTED BY THE SECRETARY OF STATE FOR INDIA,
THE ROYAL SOCIETY AND THE LISTER INSTITUTE

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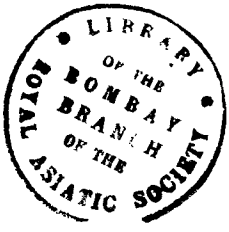

Cambridge
at the University Press
1912

Cambridge

PRINTED BY JOHN CLAY, M.A.

AT THE UNIVERSITY PRESS

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VOLUME XII DECEMBER, 1912 Supplement

REPORTS ON PLAGUE INVESTIGATIONS IN INDIA

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(*Continued from Volume XI, Plague Supplement.*)

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XLVIII. PLAGUE IN MADRAS CITY.

MADRAS City, in common with most places in the province, has suffered relatively slightly from plague. With the exception of a single epidemic in 1905–6, plague infection has never gained a foothold in the town, despite the fact that opportunities for importation appear to have been abundant since 1896, when plague first began to prevail in Bombay. The present enquiry was undertaken in order to throw light on this apparent immunity. Reducing the matter to its simplest terms, Madras City may be presumed to have escaped infection either because plague cannot reach the place or because the circumstances obtaining there are unfavourable to the propagation of imported infection. The practical part of our investigations has been for the most part directed towards an examination of this latter question especially as regards the circumstances of the rat and flea population of the town.

1. *Introduction.*

Madras is the third largest city in India and has a population of a little over half a million people (509,346). It is built in straggling fashion on a strip of land nine miles long and of an average breadth of about three miles. It is low-lying and flat. Though possessing little more than half the population of Bombay, the area of Madras is five square miles larger than the area of Bombay. It is quite unlike the other presidency towns of India and can best be described as a collection of small towns and villages separated one from the other by considerable tracts of open country. In some of the native quarters of Madras there is much crowding—Georgetown, Triplicane, Chintadripetta, for example, are very densely populated. In the middle of Georgetown the number of persons living in each inhabited house is thirteen. Single storied houses are the rule; double storied dwellings are common in some portions of the town.

It is difficult in a few words to convey any idea of the house construction in the native quarters of a city such as Madras when one sees all types of dwelling from the simple mud hut with mud floor and tiled or thatched roof to the solidly built two storied dwelling

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of the well-to-do inhabitants. Let it suffice to say that, from the point of view of rats and plague, the best houses, built as they are on high stone plinths, would be difficult to improve on, whereas the poorer houses afford unlimited shelter for rats. There certainly appears to be nothing in the construction of large portions of the town that can explain its immunity from plague, and, as we shall presently see, rats are present in abundance everywhere.

Risk of importation of plague by sea. Madras is a seaport of importance and ranks fifth among the Indian ports in the value of its trade. Imports exceed exports in value. Most of the imports come from the United Kingdom (70 per cent.), but a not inconsiderable amount of rice comes from Rangoon, with which place Madras is in close communication by sea. There is a large passenger trade between these two ports, and Rangoon, on both these counts, is a constant menace to Madras owing to the fact that plague is usually prevalent in the former port. Madras is an artificial harbour and until recently afforded no facilities for boats of large burthen coming alongside the wharfs. The ships are anchored some way from the quays and the cargoes are discharged into small boats in which they are taken ashore. This method of disembarking cargo diminishes, though it does not obliterate, the risk of importing plague-infected rats with grain. The rice is imported packed in gunny bags, and stored for varying periods of time in large godowns close to the docks, until disposed of by the importers. Third class passengers by steamer from Rangoon have their luggage opened and disinfected by heat. They are not compelled to submit the clothes they are wearing to disinfection. All passengers from Rangoon are passported as described below.

Risk of importation of plague by rail. Two railway systems, the 'Madras and Southern Mahratta" and the "South Indian," link up Madras with the rest of the Indian Peninsula. A northern line links up Madras with Calcutta, a southern line with Tuticorin, a north-west line with Bombay and a south-west with the west coast of the Presidency and Mysore. It is the last mentioned line that is the chief danger to Madras, for it is by means of it that the city is brought into closest communication with districts that are always plague-infected.

Most of the grain and pulse that is imported by land comes from districts that are free from plague, viz. Ganjam, Vizagapatam, Godavari, Nellore, Chingleput and North Arcot. Most of the risk, therefore, that Madras City runs of becoming plague-infected is by railway passenger

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traffic. To meet this risk all passengers arriving in Madras from plague-infected districts are passported. Every passenger is bound to take out a passport and has to report himself every day for a period of ten days to one of the passport examining offices in the city. No disinfection of any of the passengers' luggage is carried out, and merchandise travels unrestricted. Associated with the passport system is a very complete and vigorous sanitary organisation.

As an illustration of the exposure of Madras City to plague infection, we may note its relations with Bangalore in the native state of Mysore. Bangalore is distant from Madras about 190 miles and the passenger traffic between the two places is extensive. In the years 1901—1909, in a population of about 70,000, there were 15,400 deaths from plague, and in no month during that period did the number of plague deaths in Bangalore fall below 10.

2. *Plague in Madras City.*

Madras City has suffered from one mild outbreak of plague. Happily this occurred on the outskirts of the town, where it was possible to deal with it energetically. The following description of the outbreak is from a report written at the time by Major Ross and filed in the office of the Health Department of the Madras Municipality :—

Indigenous plague was first discovered in Madras on 20th January, 1905. On this date, the attention of a Sanitary Inspector having been drawn to the more than usual number of deaths in a fishing village (No. 1) on the northern outskirts of the town, a careful house-to-house enquiry was made, with the result that three females were found suffering from sloughing "ulcers" in the groin. These cases were diagnosed as plague, and were of course at once isolated.

The village consisted of 118 huts, with a population of 613, and was situated on the sea coast, with immediately on its rear the Mauritius-Fiji Emigration Depot enclosed on that and two other sides by high brick walls. A careful search was made in the infected village for dead or live rats, but none could be found in the huts. On the road between the village and the depot wall a dead rat was found on the 22nd January, and on passing the same spot about two minutes afterwards another rat some time dead was found, having evidently been thrown there by someone, most likely from inside the depot. Enquiries were at once made of the Depot Superintendent, and he admitted that rats had been dying within the depot, but he did not know of what cause or how they were disposed of. The rats on bacteriological examination at Guindy were pronounced plague infected and the depot was at once evacuted, though strangely enough no human plague cases were ever reported from amongst the emigrants with whom the depot was at the time crowded. Whether the emigration depot with its hundreds of coolies from plague-infected areas or the fishing village with its harbour-working

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population was the first to become infected was impossible to prove, as the first human case had occurred a fortnight before discovery and rat infection in a place where the sweeper was allowed to dispose of the dead rats by throwing them over the wall into the village may have been going on for any length of time.

The fishermen were at once moved into a new village provided by the Municipality a few hundred yards distant and the old village was burned down. A line of coolies was placed around the village during the burning to prevent the escape of rats, but as the work was done after dark it is just possible that some may have escaped. Just before evacuation on 24th January, 1905, another case of plague was discovered in the village, and none have occurred since, though they have long ago been allowed to reoccupy the old site. No further cases occurred in the neighbourhood until 18th February, 1905, when a very suspicious death occurred in a small village (No. 2) just south of and a few yards distant from No. 1, and on 23rd February two cases were discovered in another village (No. 3) a few yards west of No. 2. On 24th February another case occurred in No. 2 village, and on 24th and 25th three cases occurred in village No. 3. These villages were similarly dealt with to No. 1, and during the burning down coolies were posted to prevent the escape of rats; one, however, did make good his escape.

Immediately it was discovered that the rats had become infected, a rat destruction campaign was begun in and around the infected area. Unfortunately only a small percentage of those killed were bacteriologically examined and no further plague infection of rats was discovered. The campaign was however kept up with varying success. By the offering of rewards a fair bag, reaching as many as 100 rats of sorts daily, was collected in and around the infected area, though many of them had been killed in far away parts of the town.

Owing to the imposition of restrictions on the liberty of the people by the drawing of a cordon round well wide of the infected area, and requiring persons desirous of leaving the area to take out passes or passports, their co-operation ceased. The rat bag dwindled to insignificant proportions in spite of the doubling of the reward.

Until 30th March, 1905, no plague cases were discovered, and as we had now been 33 days clear and no infected rats had been found, we naturally began to feel hopeful. But on 1st April a suspicious case was discovered in a village several hundred yards north of the originally infected village No. 1. This case, however, was afterwards pronounced to be probably filariasis, and nothing of a suspicious nature was ever afterwards discovered in the village, although no special measures except rat destruction were carried out therein.

On 21st April a case of plague was discovered in Handcock Parcherry, a pariah village about three-quarters of a mile from and south of villages Nos. 1, 2 and 3; to reach this Parcherry a large fishing village, No. 4, and another christian Parcherry, No. 5, were passed over or seemingly so. Three more cases of plague occurred in Handcock Parcherry between 21st April and 8th May. A short distance from the Parcherry half a dozen huts were erected, and every 24 hours six families were moved into these whilst their own huts were being disinfected. Evacuation of their own huts for a night was strictly insisted upon, and during this night commonsense rat exterminator was laid in each of the evacuated huts,

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resulting in a fair total bag. Two more cases of plague occurred in this Parcherry on 26th June, the patients being visitors from a country village who were said to have arrived only three days previous to their attack. No cases have occurred in this village since and no infected rats have been found, though of late a large number have been killed here and all examined.

On 5th May a case was discovered in village No. 5, and two days afterwards this patient's wife developed the disease. On 11th June another case was discovered in this village and no cases have occurred since. The village was evacuated, but thanks to the intelligence and energy of the headman, rat destruction or attempted rat destruction has from the first been carried out here with vigour and is still kept up.

Village No. 4, known as Cassimode Kuppam, had long been under suspicion. The inhabitants—harbour coolies and fishermen—are a particularly illiterate and ignorant lot. Illness of every description was most carefully concealed, and rat catching in their village they would neither do themselves nor permit others to do. Several suspicious deaths had occurred, but no buboes were discovered on the corpses. On 20th May a female of the village was found suffering from fever and was removed for observation to the health camp. The following day she developed pneumonia, and microscopical examination of the sputa revealed abundant plague bacilli. Eight cases of plague occurred here between this and 18th August. The village was dealt with as Handcock Parcherry had been, but with the inhabitants opposing, concealing and obstructing at every step. However, no plague cases were discovered during the next four months and the Special Plague Staff was dispensed with. Again we had occasion to feel hopeful, though the fact that we could get no rats caught in village No. 4 left us in the dark with regard to what might be going on amongst them. The rats had certainly not been cleared out of this village, and it was reasonable to suppose that plague infection amongst them (if they had ever been infected) had not died out. The village was most carefully watched, and on 18th December, 1905 (after an interval of four months), a suspicious death was reported. On examination of the corpse an inguinal bubo was discovered. In this village, in January 20 cases occurred, in February 36, and up to 16th March last four more cases occurred in this and the blocks of huts between villages Nos. 4 and 5.

Rat destruction, which had practically ceased with the abolition of the Special Plague Staff, was renewed with vigour in and around the infected area from 18th December. From every rat caught, except those in an advanced state of decomposition, a spleen smear was made and microscopically examined. The first infected rat in this campaign was found on 29th December, 1905, in the area between villages Nos. 4 and 5. In the month of January 21 infected rats were found. 22 infected rats were found in February and two in March on the 10th of the month. All from an area which corresponded exactly—to an inch with the area in which human plague cases occurred. Since the 10th of March no infected rats have been found, and since 16th March there have been no plague cases amongst the people.

Altogether from 18th December to 18th April, 1888 rats were caught in and around the infected area, many having been brought from considerable distances outside, and 46 were found infected.

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The evacuation of village No. 4 was begun in December, as it was hopeless attempting to clear the rats out of it while the people remained; commonsense rat poison was laid in each hut for several nights after evacuation, and the total given above does not nearly represent the total of rats killed by the poison or which died perhaps of plague, as on shifting the roofs of the huts numbers of dried up rat carcases were found under the tiles or leaves.

With the evacuation of the village it was feared that though we were doing the best for the people of this particular village we would almost certainly drive the rats that escaped poisoning and with them the infection to adjacent villages, and this is what actually occurred to a limited extent. The infection gradually spread westwards until brought up by village No. 5, in which, as I have said, the people willingly co-operated with us in our campaign against rats.

Re-occupation of the evacuated area is now being gradually carried out, and by 1st May it is hoped all the people may be permitted to return to their permanent abodes, when their state of health will for several months be anxiously watched.

Since the close of this epidemic, no indigenous plague has been known to occur in rats or men in Madras City.

3. *The climate of Madras City.*

The climate of Madras City does not appear to offer any definite obstruction to the prevalence of plague. The following table gives the mean monthly temperature and humidity and, for comparison, the corresponding figures for three heavily infected towns.

TABLE I.

	Madras City		Bangalore		Belgaum		Bombay	
	Temperature	Humidity	Temperature	Humidity	Temperature	Humidity	Temperature	Humidity
January	75·1° F.	73 %	**68·5° F.**	**78 %**	69·9° F.	65 %	**73·0° F.**	**71 %**
February	76·7	73	**72·7**	**70**	72·8	59	**74·8**	**73**
March	80·0	74	**77·7**	**66**	77·1	55	**79·8**	**71**
April	84·0	74	81·2	72	79·2	57	**82·2**	**74**
May	86·7	67	80·2	76	77·7	66	86·2	72
June	86·4	62	75·8	81	72·6	80	83·8	82
July	84·5	65	73·8	84	70·1	91	80·2	88
August	83·3	70	73·7	85	**69·5**	**91**	80·8	85
September	83·0	72	**73·6**	**86**	**70·3**	**83**	79·5	86
October	80·6	78	**73·3**	**83**	**72·3**	**73**	80·6	80
November	77·5	79	**70·6**	**78**	**70·8**	**63**	79·5	70
December	75·5	77	**68·4**	**78**	**69·4**	**59**	76·1	76

The heavy type indicates the ordinary months of epidemic plague.

These figures show that though the summer months are hot and dry (cf. vol. VIII. p. 279), the conditions appear to be not unfavourable for plague from November to February.

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4. *The rats of Madras.*

For several years past the Corporation of Madras City have undertaken rat destruction on a small scale. The methods they were adopting when the Commission started their observations in November, 1909, consisted in offering a reward of three pies (one farthing) for each rat brought in. Since this method of rat destruction was commenced in February, 1906, 1,220,686 rats have been destroyed. A staff of twenty-five peons were employed at collecting stations scattered at various places throughout the city whose duty it was to pay out each morning the rewards for rats received, affix a label to each rat on which was written the address of the house in which the rat was caught, and forward the rats so obtained to a central depot, where an Assistant Surgeon in charge of the operations checked the number of rats so brought in, and made notes of the species. All the rats received at the depot were dead and often in an advanced stage of decomposition. Most of the rats were caught with spring traps or had been killed with sticks, the bandicoots being almost exclusively obtained in the latter way. Very few rats were caught in ordinary wire or wooden traps. An examination of the methods employed demonstrated that little reliance could be placed on the accuracy of the addresses affixed to each rat. It was obvious that such methods, though they gave an idea of the species and relative numbers of the different species of rats that were present in the city, did not give us accurate enough information of the species of rats that frequented the houses of Madras, and this information was to us of prime importance.

Arrangements were therefore made for systematic trapping of portions of the city. In selecting sections of the city for our observations, we were guided more by the density of the human population than the presence of other factors favourable or otherwise to rat infestation. In other words we paid special attention to those portions of the town in which plague, if it ever did come to Madras, would be most likely to give rise to most serious difficulty and trouble. We also paid attention to those portions of the town in the near vicinity of the docks and goods depots of the railway lines, for it is in these localities that one would expect an epizootic to start if ever the city became infected. Our methods varied but little from those we have adopted in other towns, which have been described at length

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in previous volumes of our reports. The trapping peons were in charge of an overseer, and a Sanitary Inspector was placed in charge of all the trapping operations.

Careful note was made as heretofore of the addresses of all houses trapped and those in which rats were caught. A daily examination was made of the rats caught and the fleas that they harboured. The results of our trapping and rat examination are set forth in tables II and III.

TABLE II. *Showing the number of rats caught in monthly periods.*

	rattus	Mice	Musk rats	Bandicoots	Total	Traps set	Rats per 100 traps	*rattus* per 100 traps
December 1909	1939	1386	129	28	3482	4949	67	**39**
January 1910	1965	1369	95	34	3463	4660	74	**42**
February	1741	1457	113	33	3344	4680	71	**37**
March	1236	1445	96	11	2788	3470	80	**36**
April	1059	1196	81	7	2343	3288	71	**32**
May	1437	1337	133	34	2941	4856	61	**30**
June	1378	1276	114	38	2806	5200	54	**26**
July	1302	1107	86	34	2529	5200	49	**25**
August	1391	1435	87	28	2941	5718	51	**24**
September	1593	1355	74	32	3054	5855	52	**27**
October	1389	1488	123	37	3037	5280	58	**26**
November	1233	1638	122	23	3016	5415	56	**23**
Total	17,663	16,489	1,253	339	35,744	58,571	61·0	**30·2**
	49·4 %	46·1 %	3·5 %	1·0 %	100 %			

TABLE III. *Showing the data regarding the breeding of Mus rattus.*

	rattus dissected	Adult females	Pregnant females	Percentage of adult females pregnant	Average number of foetuses per pregnant female	Young rats	Percentage of young rats
December 1909	1694	653	188	**28·8**	5·3	588	34·8
January 1910	1573	611	181	**29·6**	5·3	498	31·7
February	1246	482	172	**35·6**	5·5	419	33·6
March	1166	442	134	**30·3**	5·1	458	**39·3**
April	781	333	103	**30·9**	5·4	274	35·0
May	692	259	59	22·8	5·0	235	33·9
June	877	347	96	27·6	5·1	303	34·6
July	688	207	54	26·1	5·0	251	**36·5**
August	797	269	56	20·8	5·1	312	**39·1**
September	835	291	75	25·7	5·3	337	**40·3**
October	837	294	75	25·6	5·2	281	33·7
November	766	274	65	23·8	5·3	281	**36·8**
Total	11,952	4,462	1,258	28·2	5·2	4,237	35·4

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A table is also given (table IV) which shows the number and species of rats received at the Central Municipal rat collecting station. A comparison of the table that gives the number of rats of different species brought into the Municipal depot with the table that records the result of our trapping operations for the same months, is of some interest. In the case of trapping, it will be seen that *Mus rattus* formed 49·4 % of the total catch. Of the Municipal rats brought in dead, 51 % were *Mus rattus*. When we consider the other species, a remarkable difference occurs in their

TABLE IV. *Rats brought into the Municipal rat collecting depot.*

	Bandicoots	Bandicoots per cent.	*Mus rattus*	*Mus rattus* per cent.	Mice	Mice per cent.	Musk rats	Musk rats per cent.	Other species	Other species per cent.	
Dec. 1909	4627	17·5	15062	57·0	6026	22·4	650	2·5	60	0·2	26425
Jan. 1910	4369	18·9	12706	54·9	5516	23·8	544	2·4	2	0·008	23137
Feb.	4201	21·5	10802	55·3	4099	21·0	423	2·2	7	0·03	19532
Mar.	4345	21·2	11847	57·7	3897	19·0	423	2·1	21	0·1	20533
Apr.	3895	26·2	7210	48·5	3345	22·5	380	2·6	35	0·2	14865
May	3666	27·9	6236	47·4	2873	21·8	357	2·7	31	0·2	13163
June	5424	29·5	8210	44·7	4080	22·2	633	3·4	40	0·2	18387
July	5338	26·1	10039	49·1	4556	22·3	457	2·2	40	0·2	20430
Aug.	4207	22·2	10585	55·8	3728	19·6	425	2·2	28	0·1	18973
Sept.	4379	22·4	9522	48·8	4708	24·1	880	4·5	39	0·2	19528
Oct.	6819	29·0	9993	42·5	5623	23·9	1019	4·3	50	0·2	23504
Nov.	7667	30·2	12076	47·1	5180	20·4	453	1·8	26	0·1	25402
Total	58937	24·3	124288	51·0	53631	22·0	6644	2·7	379	0·8	243879

relative frequency as recorded in the two tables. Of the rats brought in dead 24·3 % were bandicoots. Of rats caught in traps set in houses only 1·0 % were bandicoots. This difference is very readily explained. It was mentioned above that many of the rats brought into the Municipal depots were killed with sticks. The bandicoots that inhabit the open gullies that border both sides of the road in the native quarters of the town can be seen any night scampering about the roads in large numbers. They offer a much larger mark than other species of rodent, and what is more they are surprisingly easily killed with a blow. The natives have told us that a single blow is always sufficient to kill a bandicoot and that a *rattus* requires several to disable him. In the second place, the rat traps that we employed were not large enough to capture a full-grown bandicoot though a half-grown animal could have readily entered. In spite of this second

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fact, it was only on extremely rare occasions that a young bandicoot was caught in our traps. The inhabitants of the native city have frequently assured us that bandicoots enter the houses at night in numbers and that the inhabitants are frequently disturbed at night by the noise made by these large and clumsy rodents. It is strange, therefore, that we were not successful in capturing more young ones. Our traps were always placed inside the houses. We are compelled to believe that the invasion of dwelling houses with bandicoots (at any rate young ones) is an uncommon event[1].

The absence of *decumanus* from Madras is striking. The presence of the bandicoot in such large numbers has been offered as an explanation of this fact. It is difficult to believe that people who have propounded such a theory can have made a study of the habits of the bandicoot. They have endowed the bandicoot with ferocious qualities of which it is quite innocent. We have had *Mus rattus*—a very much less formidable rat than *Mus decumanus*—living in cages with bandicoots for long periods. In no instance were the *Mus rattus* molested.

Mice form 46·1 % of the total number of rodents that were caught in traps, whereas only 22 % of those brought in dead to the Municipal depots were mice. This difference is readily accounted for when the difference in the manner in which they were obtained is taken into consideration. Both sets of figures demonstrate how extremely numerous mice are in Madras, much more so than in other places where the Commission have undertaken similar observations.

The most important fact that the two tables bring out is that in Madras, as in the vast majority of Indian towns, *Mus rattus* is the predominant house rat. As far as we are able to judge from our trapping results, the infestation of Madras City with *Mus rattus* is not definitely less than in other places where we have worked which have been plague infected. Thus the average number of *rattus* per 100 traps set was 30; with similar methods of counting, except that in Madras the places where traps were set were to some extent selected (above, p. 213), in Poona the figure was 28, in Belgaum 23, in Parel 24. On the other hand in various places in Eastern Bengal and Assam, which were free from plague, from two to ten rats were caught (vol. XI. supplement, p. 187). It should perhaps, however, be noted that in Poona, after a year without any plague, the *rattus* per 100 traps rose to 42 and after two years to as many as 70. The great relative abundance of mice in Madras also suggests that the rat

[1] In Belgaum however bandicoots live in the houses: vol. x. p. 459.

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population is not really so dense as in the other places mentioned, where hardly any mice were caught.

The *susceptibility of Madras rattus to plague* has been examined on many occasions. The details are given elsewhere (below, p. 257). The result is that Madras *rattus* are exceptionally susceptible to the hypodermic injection of small doses of an emulsion of a plague rat's spleen, 95 to 100 % dying of plague as against 20 to 40 % of *rattus* from Bombay, Poona or Belgaum.

5. *The rat fleas of Madras City.*

Practically the only species of flea found on the rats in Madras City is *X. cheopis*. On one or two occasions a specimen of *C. felis* was obtained, but such an occurrence was very rare.

Table V shows the result of the flea counts carried on throughout a complete year.

TABLE V. *Showing the prevalence of fleas with meteorological data.*

	Number of *rattus* "flea counted"	Fleas caught	Fleas per *rattus*	Mean temperature	Mean humidity
December, 1909	1663	8726	**5·2**	77·8	76
January, 1910	1573	8429	**5·4**	76·5	76
February	1252	5340	**4·3**	78·0	73
March	1164	4417	3·8	80·0	76
April	781	2626	3·4	85·1	76
May	692	2713	3·9	88·5	65
June	877	1913	2·2	86·4	65
July	688	1790	2·6	84·3	74
August	797	2850	3·6	84·7	76
September	835	3259	3·9	83·1	77
October	809	2905	3·6	81·5	81
November	766	3256	**4·3**	76·6	81
Total	11,897	48,224	4·05		

The figures show that in Madras as elsewhere there is a seasonal variation in the abundance of rat fleas. *X. cheopis* was most abundant in December and January while only about half as many were found in the hot dry weather in June and July. The flea prevalence and range of seasonal variation is very similar to what was observed for *rattus* in Bombay (vol. VIII. p. 297), the maximum in Madras falling some two months earlier. But in other, and plague infected, places which have been investigated by us, though the minimum figure is about the same (two or three per rat), during the earlier part of the plague season each *rattus* has yielded on the average 19 fleas in Belgaum, 12 in Dhand, 13 in Kasel, 9 in Poona (vol. XI. supplement, p. 188), 13 in Lucknow, 18 in Cawnpore[1], as against a monthly

[1] The details will appear in a later report.

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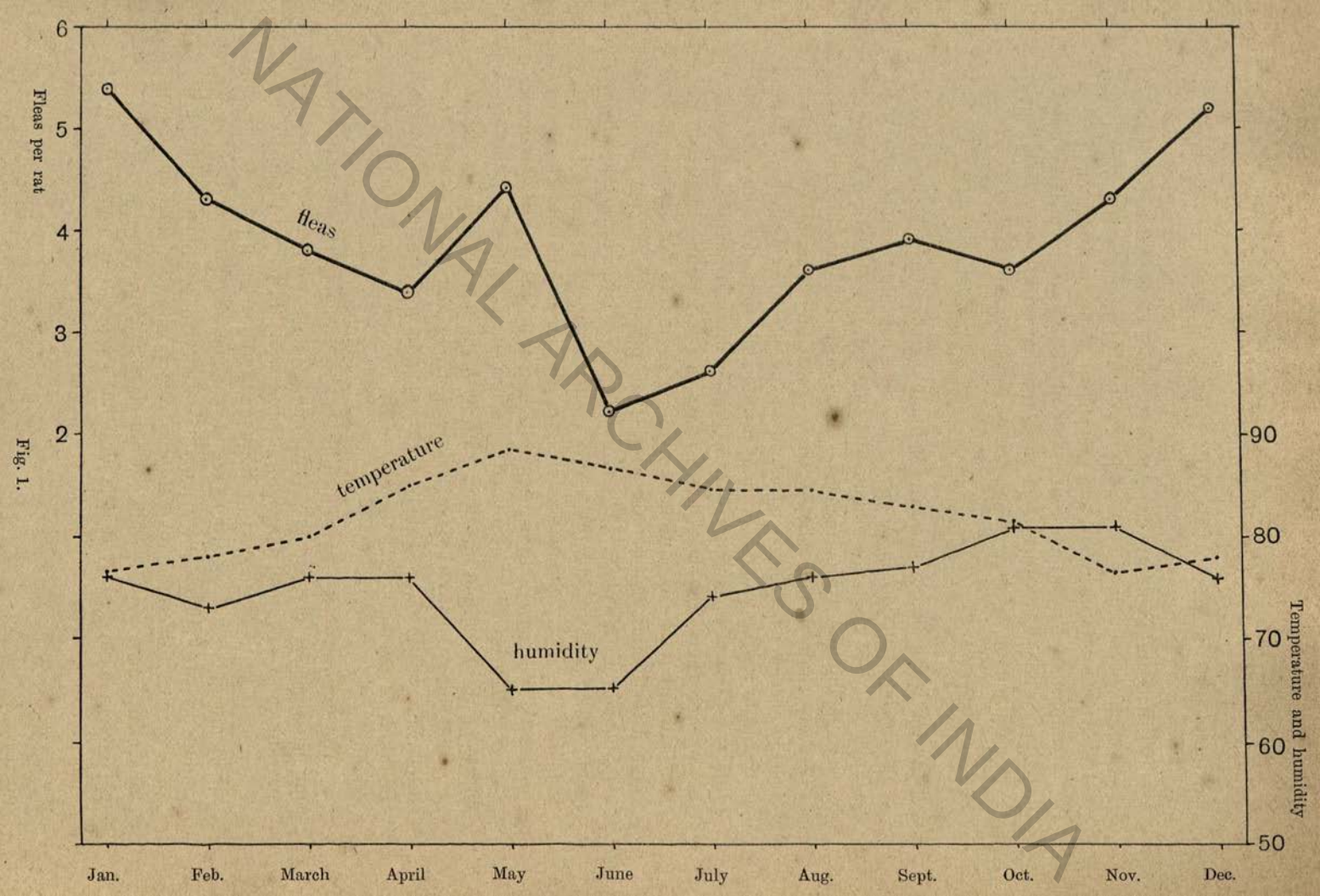

Fig. 1.

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maximum of 5 in Madras City. In Bombay, it should also be noted, on *decumanus* the fleas rise to as many as 14 per rat. In view, however, of the Bombay figures for *rattus*, it cannot be considered that the relative paucity of fleas in Madras constitutes a necessarily effective obstacle to the establishment and prevalence of plague infection.

The mice of Madras harbour relatively few fleas. On 1621 mice examined when fleas were most numerous on *rattus*, only 241 fleas were obtained, an average of one flea on seven mice (cf. vol. x. p. 526).

6. *Summary and conclusions.*

As the result of our enquiries we find therefore that the conditions of house construction in Madras City are not unfavourable to the establishment of epidemic plague, and that suitable climatic conditions prevail during the winter months. As regards the rats and rat fleas, it appears that, though neither are so plentiful as in other plague-infected places investigated by us, there are probably enough of both to maintain plague, though perhaps few enough to render implantation difficult. The rats, moreover, are exceptionally susceptible to plague. It appears, therefore, that Madras City is not immune to plague in the sense that the conditions prevailing there (as far as they have been investigated by us) are such that plague could not become established in the place. There has been no large outbreak which definitely proves this, for the single epidemic from which Madras has suffered was of a mild character. On the other hand infection was protracted and, as will have been gathered from the description given above, the outbreak was confined to a few small hamlets on the outskirts of the town and was dealt with with the most exemplary vigour and determination by the sanitary authorities, and the fact that the human mortality was relatively slight cannot under the circumstances be taken as evidence that the city is naturally an unsuitable place for plague.

The circumstances which condition the successful implantation of imported plague are probably the same as those which determine the prevalence of infection when once established. Imported plague is for example most likely to start an epidemic if it reaches a place at a time when meteorological conditions are most favourable and rat fleas most numerous. In Madras such circumstances are most favourable at a time of year (December to February) which coincides with the plague season in such a possible source of infection as Bangalore.

It seems then likely that Madras has escaped from plague because infection has been unable to reach there or has met with some

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obstruction immediately on its arrival. The possible sources from which infection might have been brought are very large, considering the abundant traffic with the infected areas in Mysore and in Bombay presidency. Assuming that infection travels about the country mostly in infected fleas associated either with such merchandise as grain or with the persons of human beings, it is clear that the likelihood of infection traversing any given distance will be proportional to the conditions being favourable to the life of the flea. Experiments have shown (see below, p. 317) that meteorological circumstances have a very large influence on the duration of life of rat fleas apart from their host, a cool moist atmosphere allowing them to survive for ten times as long as in hot dry air. Evidently therefore fleas would have some difficulty in arriving alive at any place which was surrounded by a zone of country where a high temperature, especially in conjunction with a low humidity, continuously prevailed. Madras is on the whole a hot place, but in the cooler months the temperature would apparently allow fleas to live for some considerable time though there is no definitely cold weather to afford really favourable conditions for flea importation.

So much for the influence of natural circumstances. Artificial influences are found in the passport system and its associated energetic sanitary administration[1]. As is often the case with practical sanitation where mixed experiments are made by taking various precautionary measures simultaneously, it is difficult to form an accurate estimate of the precise effect of the passport system in vogue in the province of Madras. It does not seem to discover very many cases of peripatetic plague and a fair number of infected persons appear to escape its surveillance. On the other hand its influence in preventing persons who are or may be infected from starting on any journey is very likely considerable.

In view of the above results it seemed proper to extend the scope of the enquiry to other parts of the Madras Presidency, especially as regards the conditions which have a bearing on the facilities for the importation of infection. This investigation is now being undertaken and will shortly be completed, and we reserve any further discussion of the questions outlined above until the whole problem of plague in the Madras Presidency can be examined in detail.

[1] See on the whole question, and especially on this subject, the important papers by Col. W. G. King, C.I.E., late Sanitary Commissioner to the Government of Madras, on "The Prevention of Plague in the Madras Presidency" in the *Journal of State Medicine*, 1912.

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XLIX. STATISTICS OF THE OCCURRENCE OF PLAGUE IN MAN AND RATS IN BOMBAY, 1907—1911.

SINCE the Commission brought their enquiry into rat plague in Bombay to an end in the latter part of 1906, the collection of rats on a large scale has been continued by the Bombay City Municipal Health Department and these animals have been examined for plague at the Bombay Bacteriological Laboratory where the headquarters of the Commission is established. The figures have been published from time to time in the annual reports of the Bacteriological Laboratory by the Director of the Laboratory and in the tables below they are collected together on a uniform plan.

The data for human plague are taken from the returns of the Health Department. The rat figures have been corrected for the fact that in some weeks the collection and examination of rats was suspended for one or two days.

The results are of much interest showing as they do that the phenomena (vol. VII. pp. 759, 764 and chart) observed in 1905-6 have since been repeated year after year in Bombay City. More particularly the figures show how the rat epidemic precedes the human epidemic and how the epidemic among *M. decumanus* comes a little before that in *M. rattus*.

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1907.

Total rats examined 107,875.

		Human plague mortality		Plague infected *rattus*		Plague infected *decumanus*	
		Recorded deaths from plague	Percentage above and below mean for year	Number of rats found infected	Percentage above and below mean for year	Number of rats found infected	Percentage above and below mean for year
January	1—15	63	−76	108	−43	691	**+12**
,,	16—31	108	−59	245	**+29**	1198	**+94**
February	1—15	211	−21	331	**+75**	1412	**+129**
,,	16—28	393	**+46**	485	**+155**	1798	**+191**
March	1—15	979	**+264**	602	**+217**	2093	**+239**
,,	16—31	1455	**+441**	764	**+303**	2403	**+289**
April	1—15	1190	**+342**	491	**+159**	1441	**+133**
,,	16—30	794	**+195**	331	**+75**	867	**+46**
May	1—15	443	**+65**	159	−16	443	−28
,,	16—31	214	−20	88	−53	265	−57
June	1—15	77	−71	20	−91	104	−83
,,	16—30	46	−83	40	−79	79	−87
July	1—15	42	−84	33	−82	98	−84
,,	16—31	28	−90	18	−90	69	−89
August	1—15	20	−93	47	−75	107	−82
,,	16—31	50	−82	67	−65	157	−74
September	1—15	51	−81	79	−58	143	−76
,,	16—30	63	−76	111	−42	144	−76
October	1—15	59	−78	135	−29	160	−74
,,	16—31	43	−84	159	−16	174	−72
November	1—15	43	−84	96	−49	249	−59
,,	16—30	29	−89	66	−65	209	−66
December	1—15	26	−90	34	−82	200	−67
,,	16—31	28	−90	44	−77	299	−51
	Total	6,455		4,553		14,803	

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1908.

Total rats examined 110,512.

	Human plague mortality		Plague infected *rattus*		Plague infected *decumanus*	
	Recorded deaths from plague	Percentage above and below mean for year	Number of rats found infected	Percentage above and below mean for year	Number of rats found infected	Percentage above and below mean for year
January 1—15	20	−91	57	−59	410	−28
,, 16—31	59	−74	86	−26	656	**+16**
February 1—15	92	−59	142	**+22**	796	**+39**
,, 16—28	264	**+16**	166	**+43**	1217	**+113**
March 1—15	568	**+150**	256	**+120**	1675	**+193**
,, 16—31	914	**+302**	321	**+176**	2273	**+298**
April 1—15	942	**+315**	353	**+204**	1885	**+230**
,, 16—30	907	**+299**	308	**+164**	1336	**+134**
May 1—15	626	**+176**	221	**+90**	886	**+55**
,, 16—31	340	**+50**	143	**+28**	475	−17
June 1—15	144	−36	84	−28	211	−63
,, 16—30	62	−73	55	−54	189	−67
July 1—15	62	−73	58	−59	116	−80
,, 16—31	74	−67	71	−39	129	−77
August 1—15	77	−66	91	−22	144	−75
,, 16—31	65	−71	60	−49	153	−73
September 1—15	52	−77	35	−70	167	−70
,, 16—30	55	−76	59	−49	131	−77
October 1—15	32	−86	46	−69	120	−79
,, 16—31	32	−86	55	−53	96	−83
November 1—15	19	−91	38	−67	115	−80
,, 16—30	11	−95	32	−72	129	−77
December 1—15	15	−93	29	−75	182	−68
,, 16—31	9	−96	23	−80	221	−61
Total	5,441		2,789		13,512	

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1909.

Total rats examined 91,540.

		Human plague mortality		Plague infected *rattus*		Plague infected *decumanus*	
		Recorded deaths from plague	Percentage above and below mean for year	Number of rats found infected	Percentage above and below mean for year	Number of rats found infected	Percentage above and below mean for year
January	1—15	24	−89	46	−62	244	−39
,,	16—31	42	−80	74	−22	362	−10
February	1—15	68	−60	103	**+8**	504	**+25**
,,	16—28	209	−4	151	**+59**	766	**+90**
March	1—15	481	**+121**	231	**+144**	1199	**+198**
,,	16—31	1013	**+366**	336	**+254**	1599	**+298**
April	1—15	810	**+273**	270	**+185**	1206	**+200**
,,	16—30	994	**+358**	273	**+188**	976	**+143**
May	1—15	663	**+206**	159	**+68**	564	**+40**
,,	16—31	329	**+52**	84	−12	308	−23
June	1—15	103	−53	30	−68	133	−67
,,	16—30	56	−74	29	−70	110	−73
July	1—15	48	−78	20	−79	104	−74
,,	16—31	57	−74	29	−70	101	−75
August	1—15	69	−68	28	−71	94	−77
,,	16—31	69	−68	41	−57	75	−81
September	1—15	38	−82	37	−61	95	−76
,,	16—30	40	−81	22	−76	80	−80
October	1—15	25	−88	32	−66	111	−72
,,	16—31	20	−90	43	−55	143	−64
November	1—15	13	−94	37	−60	159	−60
,,	16—30	13	−94	64	−33	188	−53
December	1—15	12	−94	62	−34	227	−43
,,	16—31	11	−95	76	−19	297	−26
	Total	5,207		2,277		9,645	

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1910.

Total rats examined 119,456.

		Human plague mortality		Plague infected *rattus*		Plague infected *decumanus*	
		Recorded deaths from plague	Percentage above and below mean for year	Number of rats found infected	Percentage above and below mean for year	Number of rats found infected	Percentage above and below mean for year
January	1—15	22	−85	96	−22	382	−16
,,	16—31	61	−60	179	**+46**	585	**+28**
February	1—15	126	−17	207	**+68**	824	**+80**
,,	16—28	169	**+11**	246	**+100**	1008	**+121**
March	1—15	259	**+70**	194	**+58**	1005	**+120**
,,	16—31	439	**+189**	195	**+58**	1051	**+130**
April	1—15	442	**+190**	205	**+67**	1065	**+133**
,,	16—30	482	**+217**	231	**+88**	1033	**+126**
May	1—15	497	**+227**	181	**+48**	741	**+62**
,,	16—31	342	**+125**	102	−17	442	−3
June	1—15	168	**+10**	87	−29	321	−30
,,	16—30	111	−27	68	−45	276	−40
July	1—15	102	−33	96	−22	279	−39
,,	16—31	120	−21	75	−39	217	−53
August	1—15	68	−55	65	−47	193	−58
,,	16—31	65	−57	105	−14	213	−53
September	1—15	25	−83	69	−43	157	−66
,,	16—30	27	−82	87	−29	141	−69
October	1—15	33	−78	82	−33	90	−80
,,	16—31	27	−82	107	−13	148	−68
November	1—15	27	−82	81	−34	134	−70
,,	16—30	17	−89	74	−40	150	−67
December	1—15	14	−90	58	−55	175	−62
,,	16—31	18	−88	46	−63	221	−51
	Total	3,661		2,936		10,851	

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1911.

Total rats examined, 134,401.

		Human plague mortality		Plague infected *rattus*		Plague infected *decumanus*	
		Recorded deaths from plague	Percentage above and below mean for year	Number of rats found infected	Percentage above and below mean for year	Number of rats found infected	Percentage above and below mean for year
January	1—15	25	– 85	59	– 42	337	– 32
,,	16—31	85	– 49	109	**+8**	530	**+8**
February	1—15	129	– 22	155	**+54**	786	**+60**
,,	16—28	251	**+51**	204	**+102**	1140	**+132**
March	1—15	366	**+121**	184	**+82**	1336	**+172**
,,	16—31	497	**+199**	242	**+140**	1600	**+225**
April	1—15	686	**+313**	267	**+165**	1533	**+212**
,,	16—30	612	**+269**	223	**+120**	1049	**+113**
May	1—15	507	**+205**	245	**+142**	872	**+77**
,,	16—31	300	**+81**	120	**+19**	517	**+5**
June	1—15	98	– 41	56	– 44	266	– 46
,,	16—30	97	– 42	41	– 59	228	– 54
July	1—15	54	– 67	36	– 65	156	– 68
,,	16—31	58	– 65	49	– 51	167	– 66
August	1—15	22	– 87	41	– 59	137	– 72
,,	16—31	42	– 75	19	– 81	113	– 77
September	1—15	27	– 84	64	– 37	114	– 77
,,	16—30	33	– 80	47	—53	131	– 73
October	1—15	21	– 87	55	– 46	129	– 74
,,	16—31	12	– 93	43	– 57	99	– 80
November	1—15	13	– 92	43	– 58	106	– 78
,,	16—30	16	– 90	43	– 58	139	– 72
December	1—15	11	– 93	38	– 63	127	– 74
,,	16—31	30	– 82	43	– 58	191	– 61
	Total	3,992		2,436		11,803	

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L. THE DISTRIBUTION OF WHITE-BELLIED *MUS RATTUS* IN BOMBAY ISLAND.

We have previously noted the occurrence in small numbers of the white-bellied form of *Mus rattus* known as *M. alexandrinus* in Poona (vol. x. p. 521) and their occurrence in Bombay has been known since we began our observations there (vol. vii. p. 743). In the present note we give statistics showing that this form is not evenly distributed about the island, and is relatively more abundant where there is plenty of open ground. The table gives the rat figures, all the animals having been trapped alive in houses and buildings, together with the density of the population in the different wards[1] as being the most convenient expression of the conditions which seem to influence the frequency of *alexandrinus*.

Ward	A			B north			B south			C			D		
Month	Total *rattus*	*Alexandrinus*	Per mille	Total *rattus*	*Alexandrinus*	Per mille	Total *rattus*	*Alexandrinus*	Per mille	Total *rattus*	*Alexandrinus*	Per mille	Total *rattus*	*Alexandrinus*	Per mille
Jan. 1910	3621	16	4	5211	9	2	4792	31	6	5374	4	1	3900	8	2
February	3007	7	2	4607	11	2	4391	49	11	4225	1	0	3431	6	2
March	3174	29	9	4152	13	3	4461	30	7	3565	3	1	3551	8	2
April	2635	9	2	3788	3	1	4457	49	11	2740	1	0	2734	4	1
May	2147	10	4	3300	15	5	3711	21	6	2100	1	0	2422	4	2
June	2238	22	9	3873	12	3	3664	34	9	2267	7	3	2539	2	1
July	2344	16	6	3968	17	4	3851	44	11	2982	6	2	2394	9	4
August	2840	8	2	4007	9	2	4502	35	8	2947	4	1	2904	13	4
September	2872	19	6	3326	13	4	4589	32	7	2681	3	1	4286	3	1
October	2860	12	4	3567	4	1	6248	85	14	2749	5	2	4141	3	1
November	3097	19	6	4615	5	1	7019	62	9	3939	5	1	4953	10	2
Total	30,835	167	5	44,414	111	2	51,685	472	9	35,569	40	1	37,255	70	2
Population per acre	50			186			287			416			70		

Ward	E east			E west			F			G			Total		
Jan. 1910	4720	54	11	2593	4	2	3153	86	27	2171	50	23	35,535	262	7
February	4018	26	6	2192	2	1	2595	70	27	2119	43	20	30,585	215	7
March	3837	73	19	1966	0	0	2439	14	6	1664	48	29	28,809	218	8
April	3760	51	14	2000	0	0	2473	53	21	1701	36	21	26,288	206	8
May	3260	38	12	1823	0	0	1969	11	6	1122	33	29	21,854	133	6
June	3656	54	15	2550	4	2	1732	45	26	897	9	10	23,416	189	8
July	3369	53	16	3259	0	0	1633	52	32	1444	32	22	25,244	229	9
August	4262	96	23	3761	9	2	1674	72	43	1176	17	14	28,073	263	9
September	3869	48	12	3398	4	1	1987	53	27	1448	36	25	28,456	211	7
October	4013	97	24	2965	8	3	1996	89	45	1839	69	38	30,378	372	12
November	5180	76	15	3027	5	2	2392	68	28	2697	52	19	36,919	302	8
Total	43,944	666	15	29,534	36	1	24,043	613	26	18,278	425	23	315,557	2600	8·2
Population per acre	82			258			18			39					

[1] The grouping of sections into wards is not that which is now used by the Health Authority. For the sections see vol. vii. pp. 727, 778.

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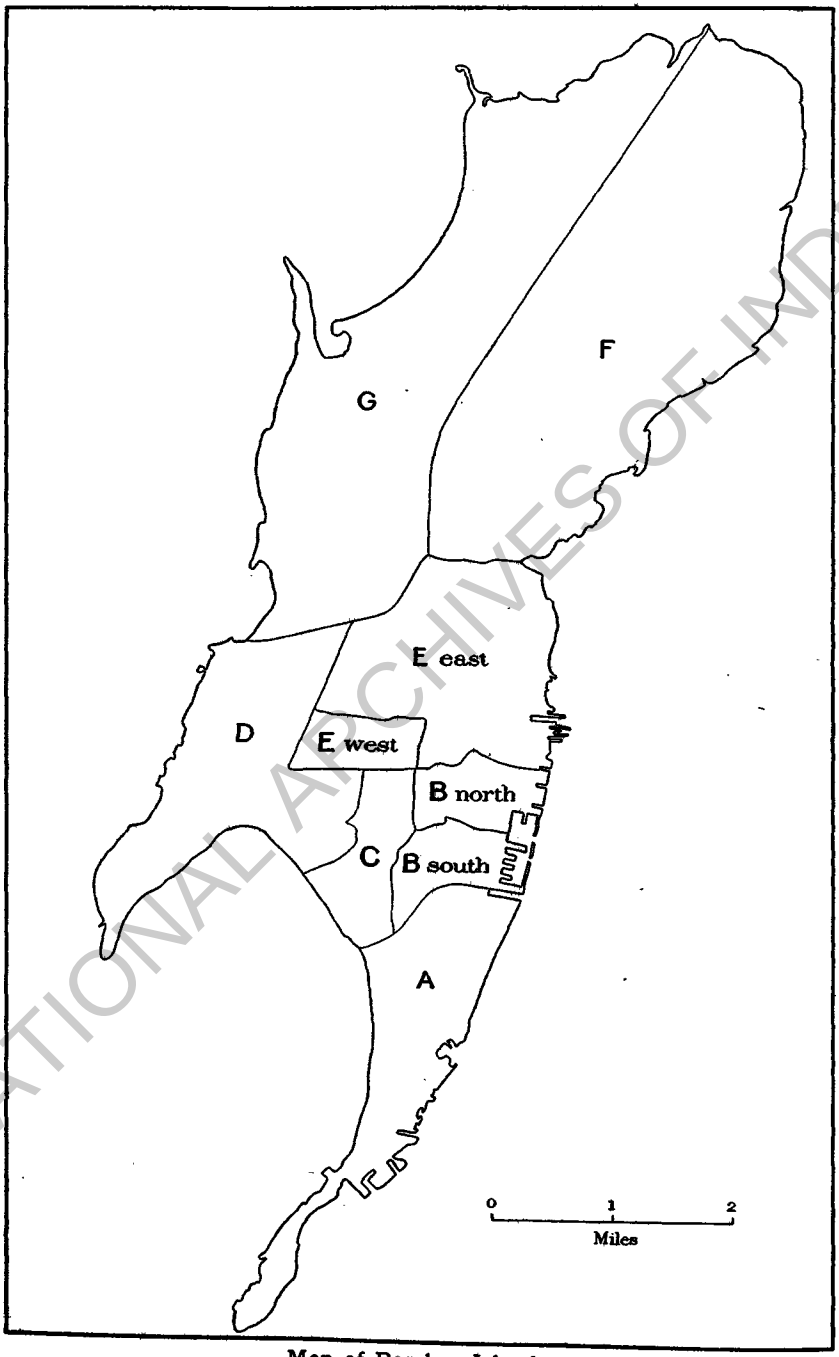

Map of Bombay Island.

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LI. THE IMMUNITY OF THE WILD RAT IN INDIA.

It is natural to suppose that the relative susceptibility or immunity of the rat to plague infection is a matter of considerable importance in the epidemiology of this disease. We have referred to the immunity of the rat to plague on several occasions, among others, for example, when discussing the factors which possibly tend to bring an epidemic of this disease to a close. We were not, however, able to bring forward any experimental evidence to show that the immunity of the rat was a factor of importance in regulating the course of an epidemic. We made several attempts to prove that rats possess a high degree of immunity at the close of an epizootic. In particular we may refer to an attempt to test this point which is mentioned in our first report on our Poona observations (vol. x. p. 523), and also to the results obtained when we tested the immunity of the survivors of the epizootics we produced in our godowns (vol. x. p. 332; this volume, p. 298).

In the Poona experiments we were confronted with great technical difficulties. We found that the varying climatic conditions at different times of the year influenced greatly the mortality among the rats under experiment; a cold night was sufficient to cause a high mortality, sick rats apparently dying from the effects of cold. Indeed we found as the result of this experiment that, in place of the immunity of Poona rats increasing as the epizootic progressed, their immunity to plague, as tested by our method of subcutaneous injection of small doses of plague bacilli, varied rather with the temperature, so that the colder the weather the greater was the mortality. Again, during the monsoon months, when the cages in which the rats were kept were liable to remain damp for days, many deaths among the rats occurred either from plague or from some other cause; it was impossible to say that plague had not been the cause of death for all the rats had been injected with living plague bacilli and we could generally isolate that organism from their tissues and organs. In short we learned that the circumstances and surroundings in which the rats were kept had a considerable influence in increasing or lowering the death rate among them. Again it was a matter of no small difficulty to find some means of regulating the dose of plague bacilli injected so as to maintain an even dose in a long series

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of experiments; two variable factors had to be regulated—the virulence of the organism and the number used in each dose. We soon learned that broth cultures were of little use for our purpose, and after a prolonged series of trials of different methods we adopted the method of spleen emulsions to be described presently. We also learned that in order to obtain more or less consistent results it was necessary to inoculate an equal number of rats of constant and standard resistance at the same time as the rats whose immunity to plague we proposed to measure. These experiments therefore, dealing as they did with many hundreds of rats, although they failed to demonstrate any increase in immunity among the Poona rats at the close of an epizootic, served a most useful purpose, for the experience gained was of great use to us in the experiments presently to be described.

In the godown experiments referred to above, in which we tested the immunity of the survivors of epizootics and compared their immunity with that of the rats which survived in the control godowns, we were forced to the conclusion that it was probable that the apparently high degree of immunity of the rats which survived the epizootics as compared with those of the control godowns was due to the death of susceptible individuals during the epizootic rather than to any immunity acquired by the rats in having suffered and recovered from the disease. This observation, together with the fact that we had found the rats caught in Belgaum very resistant to doses of plague which we knew killed a fair proportion of Bombay rats (see vol. x. p. 458), raised the question, whether the rats in places which had suffered from plague were more resistant to the disease than those caught in places which had not been invaded by it. We were confronted with the problem, were the rats in plague-infected places immune to plague because they had suffered from the disease or was this immunity a racial immunity or due to some other cause?

About this time we were making an inquiry as to the reasons why the Madras Presidency had suffered less from plague than other parts of India and for this purpose we were collecting and examining rats caught in Madras City[1], a place which had practically escaped the ravages of this disease. The occasion therefore afforded us an excellent opportunity to secure, as a standard of resistance, rats which we knew had not been exposed to infection. Arrangements were therefore made at our headquarters in Bombay to receive rats caught in Madras and Poona and to compare their immunity with that of rats caught in Bombay.

[1] See above, p. 209.

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We decided to carry out the experiment in two ways:—(1) by inoculating a large number, if possible one hundred, rats at a time from each place with the same dose of plague bacilli subcutaneously, and (2) by subjecting equal numbers of rats from the three places to infection by fleas which had fed on rats infected by plague.

The technique adopted in the first experiment was as follows. One hundred rats from Madras and Bombay and seventy-six rats from Poona were placed in separate cages, two rats in each cage. The spleen of a rat which had died of acute plague having been removed aseptically was weighed. It was then placed in a sterile mortar and ground up with a known quantity of saline solution. The larger particles of this emulsion were allowed to settle and the supernatant fluid was decanted off; to this emulsion of bacilli fresh saline was added as necessary so that one cubic centimeter of the ultimate solution contained what was regarded as the equivalent of one one-hundredth part of a milligramme of the infected spleen[1]. This we found to be the most convenient dose to use in our experiments, but occasionally we used larger doses and this was effected by decreasing the amount of saline added to the emulsion. The cages containing the rats were kept in special godowns so that they were all subjected to the same conditions. The events which occurred in this experiment are shown below. In all, while 83 % of Madras rats died of plague (the remainder of the hundred having died of other causes than plague), only 44 % of Bombay rats and 30 % of Poona rats succumbed to the disease. Madras rats therefore were highly susceptible to the disease; Bombay and Poona rats comparatively immune.

In the second experiment we attempted to find out whether the above conclusion held true when the disease was propagated among the rats by infected fleas. For this purpose 25 Bombay rats were placed in each of two godowns, in two other godowns 25 Madras rats were placed in each, and in a third pair of godowns 25 Poona rats were placed in each. Into each of the six godowns containing the rats mentioned above five inoculated rats were introduced to infect the fleas which were present in the godown. In order to estimate the number of fleas in the godowns a weekly flea count was made; this was accomplished by counting the number of fleas on a certain number of the rats in each godown and finding the average number of fleas per rat. During the course of the experiment, as inoculated rats died off, others were added

[1] In later experiments the actual number of bacilli inoculated was ascertained by plating.

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to the godowns so that five were added to each godown on the 28th January when the experiment started, five on the 12th February and three to each on the 26th February. Experiment II details the daily events occurring in the six godowns. Godowns 1 and 2 contained Bombay rats which showed an average weekly flea count of 4·2 and 2·2 respectively. Only three uninoculated rats died of plague in these godowns. Godowns 3 and 4 contained Poona rats. They showed an average weekly flea count of 4·4 and 4·7 fleas per rat respectively. Only one rat died in these godowns. Godowns 5 and 6 contained Madras rats. The flea count in these godowns was 4·3 and 5·0 respectively, and in them as many as thirty-six rats died of plague. This experiment showed, as the first had shown, that Poona and Bombay rats are, relatively to Madras rats, highly immune to infection.

In the first experiment a considerable mortality is recorded under the heading "deaths not due to plague." In judging whether a rat should be regarded as having died from plague or from other causes, no attempt was made to secure cultures, since all the animals alike had been inoculated, and we relied wholly on the naked-eye discovery of the typical post-mortem signs of plague with which we were now very familiar. The high mortality during the first few days was due partly to the rats having been recently trapped and so being unaccustomed to live in captivity and partly to the difficulty of handling wild rats for inoculation without causing them some injury. In other experiments the excess of "deaths not plague" occurred later on and was evidently not directly due to either of these factors. In our later experiments most of the early mortality was avoided by keeping the rats for a little while before inoculation and by greater technical experience; in the majority of experiments more than 100 rats were inoculated and the excess over a hundred surviving on the third day were killed. In stating the results of the experiments in numerical form it is difficult to make any very satisfactory correction for an excess of not-plague deaths. While it is evident that in experiment I, for example, the eleven rats which died on the two days following the day of inoculation, and to a less extent the four rats which died on the next day, had very little chance of dying of plague, and might reasonably be deducted from the number exposed to death; it is equally clear that, in experiment X, the eighteen rats which died from causes other than plague between the fourteenth and twenty-second days are in quite a different position, since the last plague death occurred on the twelfth day and all the rats which

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subsequently died of other causes had had just as good a chance of dying of plague as any of the others. The chief plague mortality has usually been on the third and fourth days after inoculation, and we have endeavoured to obtain a more accurate statement of the mortality by deducting the rats dying of other causes on the day of inoculation and the two days following. There are, however, comparatively few experiments (I, V, VI, VII) in which the alteration makes a material difference in the percentage mortality. Working along these lines we have completed twenty-three experiments, the details of which follow.

Experiment I.

100 Madras rats
100 Bombay rats
76 Poona rats
50 Madras mice
50 Bombay mice
} each received 1/100,000 gramme plague rat's spleen on 7 January, 1910.

Date 1910	Madras rats			Bombay rats			Poona rats			Madras mice			Bombay mice		
	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining
Jan. 7	—	—	100	—	—	100	—	—	76	—	—	50	—	—	50
,, 8	5	—	95	—	—	100	—	—	76	7	—	43	—	—	50
,, 9	6	1	88	3	1	96	—	1	75	2	—	41	7	1	42
,, 10	4	47	37	5	20	71	3	4	68	—	13	28	1	19	22
,, 11	1	24	12	6	14	51	—	4	64	—	9	19	—	2	20
,, 12	—	8	4	—	2	49	—	3	61	1	6	12	—	2	18
,, 13	—	1	3	—	3	46	—	2	59	—	1	11	—	—	18
,, 14	—	2	1	—	1	45	—	3	56	—	—	11	—	—	18
,, 15	—	—	—	—	1	44	—	1	55	—	—	11	—	2	16
,, 16	1	—	0	—	—	44	1	1	53	—	—	11	—	1	15
,, 17	—	—	—	6	—	38	—	—	53	—	—	11	1	—	14
,, 18	—	—	—	1	1	36	—	1	52	—	—	11	—	—	14
,, 19	—	—	—	1	—	35	1	2	49	—	—	11	—	—	14
,, 20	—	—	—	1	—	34	—	—	49	—	—	11	—	—	14
,, 21	—	—	—	2	1	31	1	1	47	—	—	11	—	—	14
,, 22	—	—	—	—	—	31	1	—	46	—	—	11	—	—	14
,, 23	—	—	—	—	—	31	—	—	46	—	—	11	—	—	14
,, 24	—	—	—	2	—	29	—	—	46	—	—	11	—	—	14
Totals	17	83	0	27	44	29	7	23	46	10	29	11	9	27	14

Percentages dead of plague:

Madras rats	...	83 % (93 %*)
Bombay rats	...	44 % (45 %*)
Poona rats...	...	30 %
Madras mice	....	58 % (71 %*)
Bombay mice	...	54 % (63 %*)

* Corrected for excessive not-plague mortality.

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Experiment II.

50 Madras rats, 50 Bombay rats and 50 Poona rats put into godowns on 28 January, 1910. Into each godown were placed 5 rats inoculated with plague on 28 January, 5 more on 12 February and 3 more on 26 February. The deaths from plague were as follows:

Date, 1910	Bombay rats		Poona rats		Madras rats	
	Godown 1	Godown 2	Godown 3	Godown 4	Godown 5	Godown 6
28th Jan. —3rd Feb.	0	0	0	0	0	0
4th Feb. —10th ,,	0	0	0	0	4	1
11th ,, —17th ,,	0	1	0	0	3	3
18th ,, —24th ,,	0	1	0	1	3	11
25th ,, — 3rd March	0	0	0	0	2	4
4th March—14th ,,	0	1	0	0	3	2
Totals	0	3	0	1	15	21

Percentages dead of plague:

Bombay rats	...	6 %
Poona rats ...	...	2 %
Madras rats	...	72 %

Average flea prevalence:

Godown 1	...	4·2
,, 2	...	2·2
,, 3	...	4·4
,, 4	...	4·7
,, 5	...	4·3
,, 6	...	5·0

Experiment III.

12 Madras rats ... ...
48 wild Bombay rats ... ...
48 rats bred from Bombay rats in captivity

were each inoculated with 1/20,000 gramme of plague rat's spleen on 5 May, 1910.

The wild Bombay rats and the bred rats were matched in pairs as regards size and weight.

Date	Madras rats			Wild Bombay rats			Godown bred rats		
	Dead not plague	Dead of plague	Re-maining	Dead not plague	Dead of plague	Re-maining	Dead not plague	Dead of plague	Re-maining
May 5, 1910	—	—	12	—	—	48	—	—	48
,, 6 ,,	—	—	12	2	—	46	—	—	48
,, 7 ,,	—	1	11	2	—	44	—	—	48
,, 8 ,,	—	8	3	—	5	39	—	—	48
,, 9 ,,	—	3	0	—	8	31	—	4	44
,, 10 ,,	—	—	—	—	2	29	—	1	43
,, 11 ,,	—	—	—	—	1	28	—	3	40
,, 12 ,,	—	—	—	—	1	27	—	3	37
,, 13 ,,	—	—	—	—	—	27	—	2	35
,, 14 ,,	—	—	—	—	—	27	—	2	33
,, 15 ,,	—	—	—	—	—	27	—	1	32
,, 16 ,,	—	—	—	—	—	27	—	2	30
,, 17 ,,	—	—	—	—	—	27	—	—	30
Totals	0	12	0	4	17	27	0	18	30

Percentages dead of plague:

Madras rats	...	100 %
Wild Bombay rats	...	35 % (39 %)
Bred rats ...	...	37·5 %

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Experiment IV.

100 Bhagalpur rats
100 Poona ,,
100 Madras ,,
100 Bombay ,,

were each inoculated with 1/20,000 gramme of plague rat's spleen on 2 June, 1910.

Date	Bhagalpur			Poona			Madras			Bombay		
	Dead of plague	Dead not plague	Remaining	Dead of plague	Dead not plague	Remaining	Dead of plague	Dead not plague	Remaining	Dead of plague	Dead not plague	Remaining
June 2, 1910	—	—	100	—	—	100	—	—	100	—	—	100
,, 3 ,,	—	—	100	—	—	100	—	—	100	—	—	100
,, 4 ,,	—	—	100	—	—	100	1	—	99	—	—	100
,, 5 ,,	5	—	95	1	—	99	22	—	77	—	—	100
,, 6 ,,	14	—	81	1	—	98	39	—	38	5	—	95
,, 7 ,,	14	—	67	2	—	96	14	—	24	6	—	89
,, 8 ,,	11	—	56	2	—	94	10	—	14	6	—	83
,, 9 ,,	6	—	50	0	—	94	5	—	9	2	—	81
,, 10 ,,	1	—	49	1	—	93	2	—	7	2	—	79
,, 11 ,,	5	—	44	0	—	93	1	—	6	0	—	79
,, 12 ,,	1	—	43	2	—	91	0	—	6	1	—	78
,, 13 ,,	0	—	43	1	—	90	0	—	6	1	—	77
,, 14 ,,	0	—	43	0	—	90	0	—	6	1	—	76
,, 15 ,,	0	—	43	0	—	90	0	—	6	1	2	73
,, 16 ,,	0	—	43	0	—	90	0	—	6	0	1	72
,, 17 ,,	0	—	43	0	—	90	0	—	6	1	0	71
Totals	57	0	43	10	0	90	94	0	6	26	3	71

Percentages dead of plague:

Madras rats ... 94 % (95 %)
Bombay rats ... 26 %
Bhagalpur rats ... 57 %
Poona rats ... ... 10 %

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Experiment V.

100 Madras rats ...
100 Poona rats ...
100 Bombay rats ...
27 Madras bandicoots
were each inoculated with 1/20,000 gramme of plague rat's spleen on 8 July, 1910.

Date	Madras			Poona			Bombay			Bandicoots		
	Dead of plague	Dead not plague	Remaining	Dead of plague	Dead not plague	Remaining	Dead of plague	Dead not plague	Remaining	Dead of plague	Dead not plague	Remaining
July 8, 1910	—	—	100	—	—	100	—	—	100	—	—	27
„ 9 „	—	—	100	—	—	100	—	8	92	—	—	27
„ 10 „	5	—	95	—	—	100	5	4	83	—	—	27
„ 11 „	77	—	18	13	3	84	17	—	66	—	—	27
„ 12 „	13	—	5	12	—	72	22	—	44	8	—	19
„ 13 „	2	—	3	4	—	68	8	—	36	10	—	9
„ 14 „	—	—	3	3	—	65	5	—	31	5	—	4
„ 15 „	—	—	3	4	—	61	1	—	30	3	—	1
„ 16 „	—	—	3	2	1	58	3	—	27	—	—	1
„ 17 „	—	—	3	2	—	56	—	—	27	—	—	1
„ 18 „	—	—	3	1	—	55	—	—	27	—	—	1
„ 19 „	—	—	3	—	—	55	—	—	27	—	—	1
„ 20 „	—	—	3	1	—	54	—	—	27	—	—	1
„ 21 „	—	—	3	—	—	54	1	—	26	—	—	1
„ 22 „	—	—	3	—	—	54	—	—	26	—	—	1
„ 23 „	—	—	3	—	1	53	1	—	25	—	—	1
„ 24 „	—	—	3	—	—	53	—	—	25	—	1	0
„ 25 „	—	—	3	1	—	52	1	1	23	—	—	0
„ 26 „	—	—	3	—	—	52	—	—	23	—	—	0
Totals	97	0	3	43	5	52	64	13	23	26	1	0

Percentages dead of plague:

Madras rats ... 97 %
Poona rats ... 43 %
Bombay rats ... ... 64 % (73 %)
Madras bandicoots ... 96 %

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Experiment VI.

100 Madras rats ... } were each inoculated with 1/50,000 gramme
100 young Poona rats... } of plague rat's spleen on 9 August,
100 young Bombay rats } 1910.

Average weights:

Madras ... ... 120 grammes
Young Poona ... 30 ,,
Young Bombay ... 30 ,,

Date	Madras rats			Young Poona rats			Young Bombay rats		
	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining
Aug. 9, 1910	—	—	100	—	—	100	—	—	100
,, 10 ,,	—	—	100	11	—	89	—	—	100
,, 11 ,,	—	2	98	1	4	84	1	6	93
,, 12 ,,	—	4	94	—	5	79	—	9	84
,, 13 ,,	—	33	61	—	6	73	—	6	78
,, 14 ,,	—	29	32	—	11	62	—	3	75
,, 15 ,,	—	11	21	—	2	60	—	5	70
,, 16 ,,	—	6	15	—	5	55	—	5	65
,, 17 ,,	—	5	10	—	3	52	—	2	63
,, 18 ,,	—	3	7	—	1	51	—	6	57
,, 19 ,,	—	1	6	—	1	50	—	0	57
,, 20 ,,	—	2	4	—	0	50	—	3	54
,, 21 ,,	—	0	4	—	1	49	—	2	52
,, 22 ,,	—	1	3	—	0	49	—	0	52
,, 23 ,,	—	1	2	—	0	49	1	0	51
Totals	0	98	2	12	39	49	2	47	51

Percentages dead of plague:

Madras rats ... ... 98 %
Young Poona rats ... 39 % (44 %)
Young Bombay rats ... 47 %

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Experiment VII.

100 Madras rats ... } were each inoculated with 1/20,000 gramme of
100 young Poona rats } plague rat's spleen on 30 September, 1910.
100 young Bombay rats }

Average weights:

Madras ... ... 94 grammes
Young Poona ... 23 ,,
Young Bombay ... 23 ,,

Date 1910	Young Poona rats			Young Bombay rats			Madras rats		
	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining
September 30	—	—	100	—	—	100	—	—	100
October 1	28	—	72	4	—	96	—	—	100
,, 2	2	1	69	4	9	83	—	1	99
,, 3	—	1	68	—	5	78	—	43	56
,, 4	—	1	67	2	7	69	—	38	18
,, 5	2	5	60	3	16	50	—	9	9
,, 6	1	6	53	—	6	44	—	6	3
,, 7	2	3	48	—	4	40	—	—	3
,, 8	—	1	47	—	6	34	—	—	3
,, 9	—	—	47	—	2	32	—	—	3
,, 11	2	1	44	—	2	30	—	—	3
,, 12	—	—	44	—	—	30	—	—	3
,, 13	1	1	42	—	—	30	—	—	3
,, 14	—	—	42	—	—	30	—	—	3
,, 15	—	—	42	—	—	30	—	—	3
,, 16	—	—	42	—	—	30	—	—	3
,, 17	—	—	42	—	—	30	—	—	3
,, 18	—	—	42	—	—	30	—	—	3
,, 19	—	—	42	—	—	30	—	—	3
,, 20	—	—	42	—	—	30	—	—	3
Totals	38	20	42	13	57	30	0	97	3

Percentages dead of plague:

Madras rats ... ... 97 %
Young Poona rats ... 20 % (29 %)
Young Bombay rats ... 57 % (62 %)

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Experiment VIII.

100 Poona rats
100 Kirkee rats
100 Bombay rats
} were each inoculated with 1/5,000 gramme of plague rat's spleen on 7 November, 1910

and

100 Madras rats received one-half the dose of the same emulsion.

Date	Poona			Kirkee			Madras			Bombay		
	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining
Nov. 7, 1910	—	—	100	—	—	100	—	—	100	—	—	100
,, 8 ,,	—	—	100	—	—	100	—	—	100	—	—	100
,, 9 ,,	—	2	98	—	1	99	—	12	88	—	4	96
,, 10 ,,	—	28	70	—	50	49	—	86	2	1	27	68
,, 11 ,,	—	25	45	—	22	27	—	1	1	—	19	49
,, 12 ,,	—	9	36	—	11	16	—	—	1	—	8	41
,, 13 ,,	—	7	29	—	7	9	—	—	1	—	9	32
,, 14 ,,	—	3	26	—	2	7	—	—	1	—	3	29
,, 15 ,,	—	1	25	—	1	6	—	—	1	—	—	29
,, 16 ,,	—	1	24	—	1	5	—	1	0	—	—	29
,, 17 ,,	—	0	24	—	—	5	—	—	0	—	—	29
,, 18 ,,	—	1	23	—	—	5	—	—	0	—	2	27
,, 19 ,,	—	—	23	—	—	5	—	—	0	—	—	27
,, 20 ,,	—	—	23	—	—	5	—	—	0	—	—	27
,, 21 ,,	—	—	23	—	—	5	—	—	0	—	1	26
Totals	0	77	23	0	95	5	0	100	0	1	73	26

Percentages dead of plague:

Madras rats	...	100 %
Kirkee rats	...	95 %
Poona rats	...	77 %
Bombay rats	...	73 %

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Experiment IX.

100 Madras rats
100 Poona rats
100 Bombay rats
were each inoculated with 1/5,000 gramme of plague rat's spleen on 5 January, 1911.

Date	Madras rats			Poona rats			Bombay rats		
	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining
Jan. 5, 1911	—	—	100	—	—	100	—	—	100
,, 6 ,,	—	—	100	—	—	100	—	—	100
,, 7 ,,	—	8	92	—	3	97	—	—	100
,, 8 ,,	—	79	13	1	13	83	2	38	60
,, 9 ,,	—	10	3	—	31	52	—	23	37
,, 10 ,,	—	3	0	—	14	38	—	5	32
,, 11 ,,	—	—	—	—	11	27	—	5	27
,, 12 ,,	—	—	—	—	4	23	—	3	24
,, 13 ,,	—	—	—	—	1	22	—	1	23
,, 14 ,,	—	—	—	—	—	22	—	2	21
,, 15 ,,	—	—	—	—	1	21	—	1	20
,, 16 ,,	—	—	—	—	—	21	—	—	20
,, 17 ,,	—	—	—	—	—	21	—	2	18
,, 18 ,,	—	—	—	—	—	21	—	—	18
,, 19 ,,	—	—	—	—	—	21	—	1	17
,, 20 ,,	—	—	—	—	—	21	—	1	16
Totals	0	100	0	1	78	21	2	82	16

Percentages dead of plague:

Madras rats	...	100 %
Poona rats	...	78 %
Bombay rats	...	82 %

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Experiment X.

100 Madras rats ...
100 Bombay rats ...
100 Kirkee rats ...
100 Poona rats ...
23 Madras bandicoots

were each inoculated with 1/20,000 gramme of plague rat's spleen on 16 January, 1911.

Date 1911	Madras rats			Bombay rats			Poona rats			Kirkee rats			Madras bandicoots		
	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining
Jan. 16	—	—	100	—	—	100	—	—	100	—	—	100	—	—	23
,, 17	—	—	100	—	—	100	—	—	100	—	—	100	—	—	23
,, 18	—	3	97	—	—	100	—	—	100	—	—	100	—	—	23
,, 19	—	27	70	—	1	99	—	7	93	—	3	97	—	1	22
,, 20	—	38	32	—	5	94	—	3	90	—	9	88	—	1	21
,, 21	—	15	17	—	8	86	—	4	86	—	10	78	—	5	16
,, 22	—	6	11	—	5	81	—	5	81	—	10	68	—	9	7
,, 23	—	5	6	—	3	78	—	1	80	—	4	64	—	5	2
,, 24	—	0	6	—	3	75	—	2	78	—	2	62	—	1	1
,, 25	—	2	4	1	0	74	—	1	77	—	1	61	—	0	1
,, 26	—	0	4	—	—	74	—	1	76	—	—	61	—	—	1
,, 27	—	0	4	—	—	74	—	—	76	—	—	61	—	—	1
,, 28	—	1	3	—	1	73	—	—	76	—	—	61	—	—	1
,, 29	—	—	3	—	—	73	—	—	76	—	—	61	—	—	1
,, 30	—	—	3	3	—	70	—	1	75	—	—	61	—	—	1
,, 31	—	—	3	7	—	63	1	—	74	—	—	61	—	—	1
Feb. 1	—	—	3	—	—	63	—	—	74	—	1	60	—	—	1
,, 2	—	1	2	1	—	62	1	—	73	—	—	60	—	—	1
,, 3	—	—	2	3	—	59	4	—	69	1	—	59	—	—	1
,, 4	—	—	2	2	—	57	—	—	69	—	—	59	—	—	1
,, 5	—	—	2	—	—	57	—	—	69	1	—	58	—	—	1
,, 6	—	—	2	—	—	57	2	—	67	2	—	56	—	—	1
,, 7	—	—	2	2	—	55	2	—	65	—	—	56	—	—	1
Totals	0	98	2	19	26	55	10	25	65	4	40	56	0	22	1

Percentages dead of plague:

Madras rats ... ... 98 %
Bombay rats ... ... 26 %
Poona rats ... ... 25 %
Kirkee rats ... ... 40 %
Madras bandicoots ... 96 %

NATIONAL ARCHIVES OF INDIA

Experiment XI.

100 young Poona rats
100 young Bombay rats
100 Madras rats
} were each inoculated with 1/50,000 gramme of plague rat's spleen on 14 February, 1911.

Average weights:

Madras ... ... 110 grammes
Young Poona ... ... 40 ,,
Young Bombay ... ... 40 ,,

Date	Young Poona			Young Bombay			Madras		
	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining
Feb. 14, 1911	—	—	100	—	—	100	—	—	100
,, 15 ,,	—	—	100	—	—	100	—	—	100
,, 16 ,,	—	2	98	4	—	96	—	—	100
,, 17 ,,	—	6	92	—	10	86	—	71	29
,, 18 ,,	—	3	89	—	10	76	—	22	7
,, 19 ,,	—	5	84	—	13	63	—	5	2
,, 20 ,,	—	4	80	—	4	59	—	1	1
,, 21 ,,	—	5	75	—	6	53	—	1	0
,, 22 ,,	—	2	73	—	3	50	—	—	—
,, 23 ,,	—	2	71	—	2	48	—	—	—
,, 24 ,,	1	—	70	—	—	48	—	—	—
,, 25 ,,	—	—	70	—	—	48	—	—	—
,, 26 ,,	1	—	69	1	—	47	—	—	—
,, 27 ,,	—	—	69	—	—	47	—	—	—
,, 28 ,,	—	—	69	1	—	46	—	—	—
Totals	2	29	69	6	48	46	0	100	0

Percentages dead of plague:

Madras rats ... ... 100 %
Young Poona rats ... 29 %
Young Bombay rats ... 48 % (50 %)

NATIONAL ARCHIVES OF INDIA

Experiment XII.

100 Madras rats
100 Dacca ,,
100 Poona ,,
100 Bombay ,,
were each inoculated with 1/100,000 gramme of plague rat's spleen on 24 February, 1911.

Date 1911	Madras rats			Dacca rats			Bombay rats			Poona rats		
	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining
Feb. 24	—	—	100	—	—	100	—	—	100	—	—	100
,, 25	—	—	100	—	—	100	—	—	100	—	—	100
,, 26	—	—	100	—	4	96	—	—	100	—	1	99
,, 27	3	85	12	—	45	51	—	16	84	—	14	85
,, 28	—	6	6	—	29	22	—	18	66	—	14	71
March 1	—	5	1	—	4	18	—	10	56	—	10	61
,, 2	—	1	0	—	6	12	2	1	53	3	5	53
,, 3	—	—	0	—	—	12	—	1	52	1	—	52
,, 4	—	—	0	—	—	12	—	1	51	—	1	51
,, 5	—	—	0	—	2	10	1	—	50	—	1	50
,, 6	—	—	0	—	—	10	—	1	49	2	—	48
,, 7	—	—	0	—	—	10	1	—	48	—	—	48
,, 8	—	—	0	—	2	8	—	—	48	—	—	48
,, 9	—	—	0	—	—	8	1	2	45	—	—	48
,, 10	—	—	0	—	—	8	—	1	44	—	—	48
,, 11	—	—	0	—	1	7	—	—	44	—	—	48
,, 12	—	—	0	—	—	7	2	—	42	—	—	48
,, 13	—	—	0	—	—	7	1	—	41	—	1	47
,, 14	—	—	0	—	—	7	—	—	41	—	—	47
,, 15	—	—	0	—	—	7	1	2	38	—	—	47
,, 16	—	—	0	—	—	7	—	—	38	1	1	45
Totals	3	97	0	0	93	7	9	53	38	7	48	45

Percentages dead of plague:

Madras rats	97 %
Dacca ,, ...	93 %
Bombay ,, ...	53 %
Poona ,, ...	48 %

NATIONAL ARCHIVES OF INDIA

Experiment XIII.

100 Madras rats
100 Dacca ,,
100 Poona ,,
100 Yeotgaon rats
100 Bombay ,,

were each inoculated with 1/50,000 gramme of plague rat's spleen on 10 March, 1911.

Date 1911	Madras rats			Dacca rats			Poona rats			Yeotgaon rats			Bombay rats		
	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining
March 10	—	—	100	—	—	100	—	—	100	—	—	100	—	—	100
,, 11	—	—	100	—	—	100	—	—	100	—	—	100	—	—	100
,, 12	—	4	96	—	—	100	—	—	100	—	1	99	—	—	100
,, 13	—	65	31	—	46	54	—	5	95	—	15	84	—	6	94
,, 14	—	25	6	—	36	18	—	5	90	—	21	63	—	9	85
,, 15	—	—	6	—	5	13	—	5	85	—	9	54	—	6	79
,, 16	—	—	6	—	3	10	—	8	77	—	9	45	—	6	73
,, 17	—	—	6	—	3	7	—	—	77	—	2	43	—	2	71
,, 18	—	2	4	—	—	7	3	2	72	—	1	42	—	1	70
,, 19	—	1	3	—	—	7	—	2	70	—	3	39	—	—	70
,, 20	—	—	3	—	1	6	2	1	67	—	2	37	—	4	66
,, 21	—	—	3	—	—	6	5	—	62	—	1	36	—	—	66
,, 22	—	—	3	—	3	3	2	2	58	—	3	33	—	1	65
,, 23	—	—	3	—	—	3	3	—	55	—	2	31	—	1	64
,, 24	—	—	3	—	—	3	—	1	54	1	—	30	1	—	63
,, 25	—	—	3	—	—	3	2	—	52	—	—	30	1	—	62
,, 27	—	1	2	—	—	3	2	—	50	—	1	29	2	—	60
,, 29	—	—	2	—	—	3	2	—	48	—	—	29	—	—	60
April 1	—	—	2	—	—	3	—	—	48	—	—	29	2	—	58
Totals	0	98	2	0	97	3	21	31	48	1	70	29	6	36	58

Percentages of rats dead of plague:

Madras rats	98 %
Dacca ,, ...	97 %
Poona ,, ...	31 %
Yeotgaon rats	70 %
Bombay ,, ...	36 %

NATIONAL ARCHIVES OF INDIA

Experiment XIV.

100 Madras rats ... ...
50 Chit Baragaon rats ...
100 Bombay rats ... ...
} were each inoculated with 1/100,000 gramme of plague rat's spleen on 22 April, 1911.

Date	Madras rats			Chit Baragaon rats			Bombay rats		
	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining
April 23, 1911	—	—	100	—	—	50	—	—	100
,, 24 ,,	—	—	100	—	—	50	1	—	99
,, 25 ,,	—	19	81	—	—	50	—	—	99
,, 26 ,,	—	44	37	—	—	50	—	8	91
,, 27 ,,	—	19	18	—	1	49	—	4	87
,, 28 ,,	—	15	3	—	2	47	—	6	81
,, 29 ,,	—	1	2	—	2	45	—	1	80
,, 30 ,,	—	1	1	—	3	42	—	2	78
May 1 ,,	—	—	1	—	4	38	—	2	76
,, 2 ,,	—	—	1	—	3	35	—	—	76
,, 3 ,,	1	—	0	—	2	33	—	—	76
,, 4 ,,	—	—	—	—	—	33	—	—	76
,, 5 ,,	—	—	—	—	—	33	—	—	76
,, 6 ,,	—	—	—	1	1	31	2	—	74
,, 7 ,,	—	—	—	—	1	30	2	—	72
,, 8 ,,	—	—	—	—	—	30	1	—	71
,, 9 ,,	—	—	—	—	—	30	1	—	70
,, 10 ,,	—	—	—	—	—	30	1	—	69
,, 11 ,,	—	—	—	—	—	30	—	—	69
,, 12 ,,	—	—	—	—	—	30	—	—	69
,, 13 ,,	—	—	—	—	—	30	—	—	69
,, 14 ,,	—	—	—	—	—	30	—	—	69
,, 15 ,,	—	—	—	—	—	30	—	—	69
Totals	1	99	0	1	19	30	8	23	69

Percentages dead of plague:

Madras ... ... 99 %
Chit Baragaon ... 38 %
Bombay ... ... 23 %

NATIONAL ARCHIVES OF INDIA

Experiment XV.

100 adult Madras rats
50 young Madras rats
100 Lucknow rats
100 Cawnpore rats
100 adult Bombay rats
100 young Bombay rats
100 adult Poona rats
100 young Poona rats
were each inoculated with 1/100,000 gramme of plague rat's spleen on 18 May, 1911.

Date 1911	Madras adult rats			Madras young rats			Lucknow rats			Cawnpore rats			Bombay adult rats			Bombay young rats			Poona adult rats			Poona young rats		
	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining
May 18	—	—	100	—	—	50	—	—	100	—	—	100	—	—	100	—	—	100	—	—	100	—	—	100
,, 19	—	—	100	—	—	50	—	—	100	—	—	100	—	—	100	—	—	100	—	—	100	—	—	100
,, 20	—	3	93	—	1	49	—	—	100	1	—	99	1	1	98	1	—	99	1	—	99	1	—	99
,, 21	—	28	69	—	28	21	1	7	92	—	3	96	—	2	96	—	3	96	—	1	98	—	3	96
,, 22	—	35	34	—	13	8	—	8	84	—	1	95	—	4	92	—	7	89	—	7	91	—	2	94
,, 23	—	13	21	—	2	6	—	4	80	—	2	93	—	1	91	—	5	84	—	5	86	—	12	82
,, 24	—	4	17	—	4	2	—	4	76	—	2	91	—	2	89	—	8	76	—	4	82	—	8	74
,, 25	—	5	12	—	2	—	—	4	72	—	3	88	—	4	85	—	6	70	—	2	80	—	2	72
,, 26	—	1	11	—	—	—	—	1	71	—	—	88	—	2	83	—	—	70	—	2	78	—	2	70
,, 27	—	1	10	—	—	—	—	2	69	—	1	87	—	1	82	—	—	70	—	2	76	—	2	68
,, 28	—	2	8	—	—	—	—	2	67	—	1	86	—	—	82	2	1	67	—	1	75	3	—	65
,, 29	—	2	6	—	—	—	—	—	67	1	—	85	—	—	82	—	1	66	—	—	75	1	1	63
,, 30	—	1	5	—	—	—	—	1	66	—	—	85	—	2	80	—	1	66	—	—	75	—	1	62
,, 31	—	—	5	—	—	—	—	2	64	—	1	84	—	—	80	—	2	64	2	—	73	1	1	60
June 1	—	—	5	—	—	—	—	—	64	1	—	83	—	—	80	3	1	60	—	—	73	—	—	60
,, 2	—	—	5	—	—	—	—	—	64	1	—	82	2	—	78	1	—	59	—	—	73	2	1	57
,, 3	—	—	5	—	—	—	—	—	64	—	1	81	—	—	78	—	—	59	—	—	73	—	—	57
,, 4	—	—	5	—	—	—	1	—	63	—	—	81	—	—	78	1	—	58	—	—	73	1	—	56
,, 5	—	—	5	—	—	—	—	1	62	—	—	81	—	—	78	—	—	58	—	—	73	1	—	55
,, 6	—	—	5	—	—	—	—	—	62	—	—	81	—	—	78	2	—	56	—	1	72	1	—	54
,, 7	—	—	5	—	—	—	—	—	62	—	—	81	—	—	78	—	—	56	1	—	71	1	—	53
,, 8	—	—	5	—	—	—	1	1	60	—	—	81	1	—	77	—	—	56	—	—	71	—	—	53
,, 9	—	—	5	—	—	—	—	—	60	—	—	81	1	—	76	—	—	56	—	—	71	—	—	53
Totals	0	95	5	0	50	0	3	37	60	4	15	81	5	19	76	10	34	56	4	25	71	12	35	53

Percentages dead of plague:

Adult Madras ...	95 %	Lucknow ...	37 %	Adult Bombay ...	19 %	Adult Poona ...	25 %
Young Madras ...	100 %	Cawnpore ...	15 %	Young Bombay ..	34 %	Young Poona ...	35 %

NATIONAL ARCHIVES OF INDIA

Experiment XVI.

100 Madras rats
100 Madura ,,
75 Cawnpore rats
75 Vaniyambadi rats
100 Bombay rats (adult)
50 ,, ,, (young)
50 Poona ,, ,,

were each inoculated with 1/100,000 gramme of plague rat's spleen on 21 June, 1911.

Date, 1911	Madras			Madura			Cawnpore			Vaniyambadi			Bombay (adult)			Bombay (young)			Poona (young)		
	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining
June 22	—	—	100	—	—	100	—	—	75	—	—	75	—	—	100	—	—	50	—	—	50
,, 23	—	3	97	—	—	100	—	—	75	—	—	75	—	—	100	—	—	50	—	—	50
,, 24	—	55	42	—	49	51	—	2	73	—	34	41	1	11	88	—	1	49	—	3	47
,, 25	—	27	15	—	37	14	—	2	71	—	23	18	—	7	81	—	5	44	—	3	44
,, 26	—	9	6	—	10	4	—	3	68	—	10	8	1	5	75	—	3	41	—	2	42
,, 27	—	3	3	—	2	2	—	2	66	—	4	4	3	11	61	4	12	25	—	2	40
,, 28	—	2	1	—	—	2	1	2	63	—	1	3	2	4	55	1	—	24	—	1	39
,, 29	—	—	1	—	—	2	1	1	61	—	—	3	—	1	54	—	1	23	—	—	39
,, 30	—	—	1	—	—	2	3	1	57	—	1	2	2	1	51	—	1	22	—	2	37
July 1	—	—	1	—	—	2	—	—	57	—	—	2	—	2	49	—	1	21	—	1	36
,, 2	—	—	1	—	2	0	2	—	55	—	1	1	—	3	46	—	—	21	—	—	36
,, 3	—	1	0	—	—	0	14	1	40	—	1	0	1	—	45	7	—	14	6	—	30
,, 4	—	—	0	—	—	0	3	—	37	—	—	0	1	—	44	—	—	14	—	—	30
,, 5	—	—	0	—	—	0	—	1	36	—	—	0	5	—	39	—	—	14	4	—	26
,, 6	—	—	0	—	—	0	—	—	36	—	—	0	4	—	35	—	—	14	3	—	23
,, 7	—	—	0	—	—	0	1	—	35	—	—	0	3	—	32	1	—	13	1	—	22
,, 8	—	—	0	—	—	0	—	—	35	—	—	0	—	—	32	—	—	13	—	—	22
,, 9	—	—	0	—	—	0	1	—	34	—	—	0	—	—	32	—	—	13	—	—	22
,, 10	—	—	0	—	—	0	—	—	34	—	—	0	1	—	31	—	—	13	—	—	22
Totals	0	100	0	0	100	0	26	15	34	0	75	0	24	45	31	13	24	13	14	14	22

Percentages dead of plague:—Madras 100 %, Madura 100 %, Cawnpore 20 %, Vaniyambadi 100 %, adult Bombay 45 %, young Bombay 48 %, young Poona 28 %.

NATIONAL ARCHIVES OF INDIA

Experiment XVII.

100 Madras rats
100 Lucknow rats
100 Cawnpore rats
100 Bansdeh rats
100 Nagpur rats
100 Poona rats
100 Bombay rats
50 Bombay rats bred in godowns
25 rats bred from the survivors of godown epizootics (see below, p. 292)

were each inoculated with 1/100,000 gramme of plague rat's spleen (=26,000 plague bacilli) on 2 August, 1911.

Date, 1911	Madras			Lucknow			Cawnpore			Bansdeh			Nagpur			Poona			Bombay			Bred in godowns			Bred from survivors of epizootics		
	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining
Aug. 3	—	—	*100*	—	—	*100*	—	—	*100*	—	—	*100*	—	—	*100*	—	—	*100*	—	—	100	—	—	50	—	—	25
,, 4	—	*3*	*97*	—	2	98	—	—	100	—	*5*	*100*	—	1	99	—	—	100	—	—	100	—	—	50	—	—	25
,, 5	—	27	70	1	3	94	—	1	99	—	5	95	—	19	80	—	1	99	—	1	99	—	1	49	—	—	25
,, 6	—	44	26	2	11	81	—	9	90	—	9	86	—	47	33	—	1	98	—	1	98	—	3	46	1	—	24
,, 7	—	16	10	—	3	78	—	1	89	1	10	75	—	13	20	—	7	91	—	7	91	—	1	45	—	—	24
,, 8	—	4	6	—	7	71	—	2	87	—	1	74	—	6	14	—	2	89	—	2	89	—	1	44	—	—	24
,, 9	—	4	2	—	4	67	—	5	82	—	6	68	—	5	9	—	1	88	—	1	88	—	1	43	—	1	23
,, 10	—	—	2	—	—	67	—	—	82	—	4	64	—	1	8	—	4	84	—	4	84	—	—	43	—	—	23
,, 11	—	—	2	—	1	66	—	—	82	1	—	63	—	4	4	2	1	81	2	1	81	—	—	43	—	—	23
,, 12	—	—	2	2	1	63	1	—	81	1	1	61	—	1	3	2	1	78	2	1	78	—	1	42	—	—	23
,, 13	—	—	2	—	—	63	2	—	79	1	—	60	—	—	3	—	2	76	—	2	76	—	1	41	—	—	23
,, 14	—	—	2	1	—	62	—	—	79	2	—	58	—	—	3	2	1	73	2	1	73	—	—	41	—	—	23
,, 15	—	1	1	—	—	62	—	—	79	2	—	56	—	—	3	1	—	72	1	—	72	—	—	41	—	—	23
,, 16	—	—	1	—	—	62	—	—	79	1	—	55	—	—	3	—	—	72	—	—	72	—	1	40	—	—	23
,, 17	—	—	1	—	—	62	1	—	78	—	1	54	—	—	3	—	—	72	1	—	71	—	—	40	—	—	23
,, 18	—	—	1	—	—	62	—	—	78	3	—	51	—	—	3	—	—	72	—	—	71	1	—	39	—	—	23
,, 19	—	—	1	—	—	62	—	1	77	1	—	50	—	—	3	—	—	72	1	—	70	—	—	39	—	—	23
,, 20	—	—	1	—	—	62	—	—	77	—	—	50	—	—	3	—	—	72	1	—	69	—	—	39	—	—	23
,, 21	—	—	1	—	—	62	—	—	77	2	—	48	—	—	3	—	—	72	—	—	69	—	—	39	—	—	23
,, 22	—	—	1	—	—	62	—	—	77	—	—	48	—	—	3	—	—	72	—	—	69	—	—	39	—	—	23
,, 23	—	—	1	—	—	62	—	—	77	—	—	48	—	—	3	—	—	72	—	—	69	—	—	39	—	—	23
,, 24	—	—	1	—	—	62	—	—	77	—	—	48	—	—	3	—	—	72	—	—	69	—	—	39	—	—	23
,, 25	—	—	1	—	—	62	—	—	77	—	—	48	—	—	3	—	—	72	—	—	69	—	—	39	—	—	23
Totals	0	99	1	6	32	62	4	19	77	15	37	48	0	97	3	7	21	72	10	21	69	1	10	39	1	1	23

Percentages dead of plague:

Madras	...	99 %	
Lucknow	...	32 %	
Cawnpore	...	19 %	
Bansdeh	...	37 %	
Nagpur	...	97 %	
Poona	...	21 %	
Bombay		...	21 %
Godown bred from Bombay rats		...	20 %
Bred from survivors of epizootics		...	4 %

NATIONAL ARCHIVES OF INDIA

Experiment XVIII.

100 Madras rats
100 Raipur rats
60 Cawnpore rats
100 Calicut rats
100 Palghat rats
100 Poona rats
100 Bombay rats

were each inoculated with 1/100,000 gramme of plague rat's spleen (=12,000 plague bacilli) on 1 September, 1911.

Date 1911	Madras rats			Raipur rats			Cawnpore rats			Calicut rats			Palghat rats			Poona rats			Bombay rats		
	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining	Dead not plague	Dead of plague	Remaining
Sept. 1	—	—	100	—	—	100	—	—	60	—	—	100	—	—	100	—	—	100	—	—	100
,, 2	—	—	100	—	—	100	—	—	60	—	—	100	—	—	100	—	—	100	—	—	100
,, 3	—	1	99	—	—	100	—	—	60	—	2	98	—	1	99	—	—	100	—	—	100
,, 4	—	13	86	—	25	75	—	1	59	—	20	78	—	14	85	—	—	100	—	—	100
,, 5	—	44	42	—	52	23	—	2	57	—	30	48	—	36	49	—	5	95	—	3	97
,, 6	—	15	27	—	13	10	—	4	53	—	15	33	—	14	35	—	6	89	—	5	92
,, 7	—	12	15	—	8	2	—	2	51	—	4	29	—	12	23	—	3	86	—	5	87
,, 8	—	4	11	—	2	0	—	1	50	—	9	20	—	8	15	—	2	84	—	2	85
,, 9	—	2	9	—	—	—	—	—	50	—	4	16	—	2	13	—	1	83	—	—	85
,, 10	—	—	9	—	—	—	1	—	49	—	1	15	—	—	13	—	—	83	1	1	83
,, 11	—	—	9	—	—	—	—	—	49	—	—	15	—	1	12	—	—	83	1	—	82
,, 12	—	1	8	—	—	—	1	—	48	—	2	13	—	1	11	—	—	83	—	—	82
,, 13	—	1	7	—	—	—	1	—	47	—	1	12	—	—	11	3	—	80	1	—	81
,, 14	—	—	7	—	—	—	—	—	47	—	—	12	—	—	11	1	1	78	1	—	80
,, 15	—	—	7	—	—	—	1	—	46	—	1	11	—	—	11	—	—	78	1	—	79
,, 16	—	—	7	—	—	—	—	—	46	—	—	11	—	—	11	—	—	78	1	—	78
,, 17	—	—	7	—	—	—	—	—	46	—	—	11	—	—	11	—	—	78	1	—	77
,, 18	—	—	7	—	—	—	—	—	46	—	—	11	—	—	11	—	—	78	—	—	77
,, 19	—	—	7	—	—	—	—	—	46	—	1	10	—	—	11	—	—	78	1	—	76
,, 20	—	1	6	—	—	—	—	—	46	—	1	9	1	—	10	2	—	76	1	—	75
,, 21	—	1	5	—	—	—	—	—	46	—	—	9	—	—	10	—	—	76	—	—	75
Totals	0	95	5	0	100	0	4	10	46	0	91	9	1	89	10	6	18	76	9	16	75

Percentages dead of plague:

Madras	... 95 %	Cawnpore	... 17 %	Palghat	... 89 %	Bombay	... 16 %
Raipur	... 100 %	Calicut	... 91 %	Poona	... 18 %		

NATIONAL ARCHIVES OF INDIA

Experiment XIX.

100 Lucknow rats, 80 Cawnpore rats, 60 Banda rats, 100 Bombay rats, 90 Poona P.C. rats, 100 Poona N.P.C. rats — each received 1/100,000 gramme plague rat's spleen (=20,000 plague bacilli) on 28 October, 1911.

Date 1911	Lucknow			Cawnpore			Banda			Bombay			Poona P.C.			Poona N.P.C.		
	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining	Not plague	Plague	Remaining
Oct. 28	—	—	100	—	—	80	—	—	60	—	—	100	—	—	90	—	—	100
,, 29	—	—	100	—	—	80	—	—	60	—	—	100	—	—	90	—	—	100
,, 30	—	1	99	—	—	80	—	1	59	—	3	97	—	1	89	—	1	99
,, 31	—	7	92	—	4	76	—	21	38	—	6	91	—	5	84	—	6	93
Nov. 1	—	14	78	—	7	69	—	23	15	—	12	79	—	7	77	—	5	88
,, 2	—	5	73	—	2	67	—	6	9	—	1	78	—	2	75	—	7	81
,, 3	—	2	71	—	3	64	—	4	5	—	4	74	—	6	69	—	3	78
,, 4	—	1	70	—	—	64	—	2	3	—	1	73	—	1	68	—	1	77
,, 5	—	1	69	—	—	64	—	1	2	—	1	72	—	4	64	—	1	76
,, 6	—	1	68	2	—	62	—	1	1	2	1	69	—	1	63	—	—	76
,, 7	—	—	68	—	—	62	—	—	1	—	1	68	—	2	61	—	—	76
,, 8	1	—	67	—	—	62	—	—	1	2	—	66	—	1	60	2	—	74
,, 9	2	—	65	—	—	62	—	—	1	—	—	66	—	—	60	—	—	74
,, 10	—	—	65	—	—	62	—	—	1	1	—	65	—	—	60	1	—	73
,, 11	—	—	65	—	—	62	—	—	1	—	—	65	—	—	60	1	—	72
,, 12	—	—	65	—	—	62	—	—	1	—	—	65	—	—	60	1	—	71
,, 13	—	—	65	—	—	62	—	—	1	—	—	65	—	—	60	—	—	71
,, 14	2	—	63	—	—	62	—	—	1	2	—	63	1	—	59	2	—	69
,, 15	—	—	63	—	—	62	—	—	1	—	—	63	—	—	59	—	—	69
,, 16	—	—	63	—	—	62	—	—	1	—	—	63	—	—	59	—	—	69
,, 17	—	—	63	—	—	62	—	—	1	—	—	63	—	—	59	—	—	69
,, 18	—	—	63	—	—	62	—	—	1	—	—	63	—	—	59	—	—	69
,, 19	—	—	63	—	—	62	—	—	1	—	—	63	—	—	59	—	—	69
Totals	5	32	63	2	16	62	0	59	1	7	30	63	1	30	59	7	24	69

Percentages dead of plague:

Lucknow ... 32 %
Cawnpore ... 20 %
Banda ... 98 %
Bombay ... ... 30 %
Poona P.C. ... 33 %
Poona N.P.C. ... 24 % } 28 %

NATIONAL ARCHIVES OF INDIA

Experiment XX.

100 Madras rats
50 Lucknow rats
100 Madura rats
100 Poona rats
100 Bombay rats
100 Nagpur rats
50 Surat rats

each received 1/100,000 gramme plague rat's spleen (=18,000 plague bacilli) on 25 November, 1911.

Date 1911	Madras			Lucknow			Madura			Poona			Bombay			Nagpur			Surat		
	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance
Nov. 25	—	—	100	—	—	50	—	—	100	—	—	100	—	—	100	—	—	100	—	—	50
,, 26	—	—	100	—	—	50	—	—	100	—	—	100	—	—	100	—	—	100	—	—	50
,, 27	—	5	95	—	—	50	—	—	100	—	2	98	—	5	95	—	6	94	—	1	49
,, 28	—	16	79	—	—	50	—	24	76	—	3	95	—	5	90	—	13	81	—	2	47
,, 29	—	43	36	—	5	45	—	52	24	—	2	93	—	13	77	—	24	57	—	4	43
,, 30	—	14	22	—	2	43	—	11	13	—	3	90	—	5	72	—	26	31	—	4	39
Dec. 1	—	8	14	—	3	40	—	5	8	—	2	88	—	8	64	—	12	19	—	3	36
,, 2	—	6	8	—	1	39	—	1	7	—	2	86	—	2	62	—	8	11	—	2	34
,, 3	—	2	6	2	3	34	—	1	6	—	—	86	1	2	59	—	5	6	—	—	34
,, 4	—	1	5	—	1	33	—	—	6	—	—	86	1	1	57	—	2	4	—	1	33
,, 5	—	—	5	—	1	32	—	1	5	2	2	82	2	—	55	—	1	3	2	1	30
,, 6	—	1	4	1	—	31	—	—	5	—	1	81	2	1	52	1	—	2	—	1	29
,, 7	—	—	4	2	—	29	—	—	5	1	—	80	—	—	52	—	—	2	—	1	28
,, 8	—	—	4	1	—	28	—	—	5	3	1	76	2	—	50	—	—	2	1	—	27
,, 9	—	—	4	2	—	26	—	—	5	—	—	76	2	—	48	—	—	2	—	—	27
,, 10	—	—	4	—	—	26	—	—	5	1	2*	73	1	—	47	—	—	2	—	1*	26
,, 11	—	—	4	1	—	25	—	—	5	—	—	73	1	—	46	—	1	1	—	—	26
,, 12	—	—	4	—	—	25	—	—	5	—	—	73	—	—	46	—	—	1	—	—	26
,, 13	—	—	4	1	—	24	—	—	5	—	—	73	—	—	46	—	—	1	—	—	26
,, 14	—	—	4	—	—	24	—	—	5	—	—	73	—	—	46	—	—	1	—	—	26
,, 15	—	—	4	—	—	24	—	—	5	—	—	73	—	—	46	—	—	1	—	—	26
Totals	0	96	4	10	16	24	0	95	5	7	20	73	12	42	46	1	98	1	3	21	26

* Chronic plague.

Percentages dead of plague:

Madras	Lucknow	Madura	Poona	Bombay	Nagpur	Surat
96 %	32 %	95 %	20 %	42 %	98 %	42 %

NATIONAL ARCHIVES OF INDIA

Experiment XXI.

100 Madras rats ...
100 Bombay rats ...
80 Poona rats ... ...
75 Muzaffarnagar rats
80 Delhi Shahdara rats

each received 1/100,000 gramme plague rat's spleen (=16,000 plague bacilli) on 31 January, 1912.

Date 1912	Madras			Muzaffarnagar			Delhi Shahdara			Poona			Bombay		
	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance
Jan. 31	—	—	100	—	—	75	—	—	80	—	—	80	—	—	100
Feb. 1	—	—	100	—	—	75	—	—	80	—	—	80	—	—	100
,, 2	—	—	100	—	1	74	—	—	80	—	—	80	—	—	100
,, 3	—	12	88	—	—	74	—	—	80	—	3	77	—	4	96
,, 4	—	30	58	—	4	70	—	3	77	—	2	75	—	—	96
,, 5	—	20	38	—	4	66	—	5	72	—	2	73	—	4	92
,, 6	—	13	25	—	2	64	—	3	69	—	3	70	—	2	90
,, 7	—	10	15	—	4	60	—	7	62	—	—	70	—	5	85
,, 8	—	2	13	—	2	58	—	1	61	—	2	68	—	5	80
,, 9	—	1	12	—	1	57	—	1	60	—	1	67	—	—	80
,, 10	—	2	10	—	—	57	—	—	60	—	—	67	—	1	79
,, 11	—	2	8	1	—	56	1	—	59	2	—	65	1	—	78
,, 12	—	2	6	—	—	56	—	1	58	—	—	65	—	1	77
,, 13	—	2	4	—	1	55	—	1	57	—	1	64	—	2	75
,, 14	—	1	3	2	—	53	1	—	56	2	—	62	—	—	75
,, 15	—	—	3	1	1	51	—	1	55	1	—	61	1	—	74
,, 16	—	—	3	—	—	51	—	—	55	—	—	61	—	—	74
,, 17	—	—	3	—	—	51	—	—	55	—	—	61	—	—	74
,, 18	—	—	3	1	—	50	—	—	55	—	—	61	1	—	73
,, 19	—	1	2	—	—	50	—	—	55	—	—	61	—	—	73
,, 20	—	—	2	—	—	50	—	—	55	—	—	61	—	—	73
,, 21	—	—	2	—	—	50	—	—	55	—	—	61	—	—	73
Totals	0	98	2	5	20	50	2	23	55	5	14	61	3	24	73

Percentages dead of plague:

Madras	Muzaffarnagar	Delhi Shahdara	Poona	Bombay
98 %	27 %	29 %	17 %	24 %

NATIONAL ARCHIVES OF INDIA

Experiment XXII.

100 Madras rats
80 Coimbatore rats
100 Poona rats
66 Yeotgaon rats
25 Bellary rats
100 Madura rats
95 Podanur rats
100 Bombay rats
18 Madras rats bred in Bombay
60 Bombay rats bred in Bombay
30 Survivors from experimental epizootics (see below, p. 292)

each received 1/100,000 gramme of a plague rat's spleen (=14,000 plague bacilli) on 27 April, 1912

Date, 1912	Madras			Madura			Poona			Yeotgaon			Bellary			Coimbatore			Podanur			Bombay			Survivors			Godown bred Madras rats			Godown bred Bombay rats		
	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance
April 27	—	—	100	—	—	100	—	—	100	—	—	66	—	—	25	—	—	80	—	—	95	—	—	100	—	—	30	—	—	18	—	—	60
,, 28	—	—	100	—	—	100	—	—	100	—	—	66	—	—	25	—	—	80	—	—	95	—	—	100	—	—	30	—	—	18	—	—	60
,, 29	—	5	95	—	2	98	—	1	99	—	1	65	—	—	25	—	—	80	—	1	94	—	1	99	—	—	30	—	2	16	—	—	60
,, 30	—	27	68	—	19	79	—	1	98	—	18	47	—	3	22	—	3	77	—	23	71	—	2	97	—	—	30	—	12	4	—	2	58
May 1	—	36	32	—	32	47	—	—	98	—	17	30	—	—	22	—	8	69	—	35	36	—	8	89	—	—	30	—	2	2	—	2	56
,, 2	—	10	22	—	13	34	—	6	92	—	4	26	—	1	21	—	15	54	—	6	30	—	5	84	—	—	30	—	2	0	—	3	53
,, 3	—	5	17	—	6	28	—	1	91	—	5	21	—	—	21	—	3	51	—	7	23	—	2	82	—	—	30	—	—	—	—	3	50
,, 4	—	2	15	—	3	25	—	—	91	—	—	21	—	2	19	—	4	47	—	5	18	—	1	81	1	—	29	—	—	—	—	1	49
,, 5	—	1	14	—	1	24	—	1	90	—	1	20	—	1	18	—	9	38	—	1	17	—	2	79	—	—	29	—	—	—	—	—	49
,, 6	—	2	12	—	2	22	—	1	89	—	1	19	—	2	16	—	—	38	—	1	16	2	2	75	—	—	29	—	—	—	—	1	48
,, 7	—	1	11	—	1	21	—	1	88	—	—	19	—	—	16	—	—	38	—	—	16	—	1	74	1	—	28	—	—	—	—	—	48
,, 8	—	—	11	—	—	21	—	1	87	—	—	19	—	1	15	—	—	38	—	1	15	—	1	73	—	—	28	—	—	—	—	1	47
,, 9	—	2	9	—	2	19	—	—	87	—	4	15	—	—	15	—	2	36	—	3	12	—	—	73	—	—	28	—	—	—	—	—	47
,, 10	—	—	9	—	—	19	—	—	87	—	—	15	—	—	15	—	1	35	—	1	11	—	1	72	—	—	28	—	—	—	—	—	47
,, 11	—	2	7	—	—	19	1	—	86	—	1	14	—	—	15	—	—	35	—	2	9	1	—	71	—	—	28	—	—	—	1	—	46
,, 12	—	1	6	—	1	18	—	—	86	—	—	14	—	—	15	—	2	33	—	1	8	—	1	70	—	—	28	—	—	—	—	—	46
,, 13	—	2	4	—	1	17	1	—	85	—	—	14	—	—	15	—	—	33	—	—	8	1	—	69	—	—	28	—	—	—	1	—	45
,, 14	—	—	4	—	—	17	1	—	84	—	—	14	—	—	15	—	—	33	—	—	8	—	—	69	—	—	28	—	—	—	—	—	45
,, 15	—	—	4	—	—	17	—	—	84	—	—	14	—	—	15	—	—	33	—	—	8	—	—	69	—	—	28	—	—	—	—	—	45
,, 16	—	1	3	—	—	17	—	—	84	—	—	14	—	—	15	—	—	33	—	—	8	—	—	69	—	—	28	—	—	—	—	—	45
,, 17	—	—	3	—	—	17	—	—	84	—	—	14	—	—	15	—	—	33	1	—	7	—	—	69	—	—	28	—	—	—	—	—	45
,, 18	—	—	3	—	—	17	—	—	84	—	—	14	—	—	15	—	—	33	—	—	7	1	—	68	—	—	28	—	—	—	—	—	45
Totals	0	97	3	0	83	17	3	13	84	0	52	14	0	10	15	0	47	33	1	87	7	5	27	68	2	0	28	0	18	0	2	13	45

Madras ... 97 %
Madura ... 83 %
Poona ... 13 %
Yeotgaon ... 79 %
Bellary ... 40 %
Coimbatore ... 60 %
Podanur ... 92 %
Bombay ... 27 %
Survivors ... nil %
Madras (bred) ... 100 %
Bombay (bred) ... 22 %

NATIONAL ARCHIVES OF INDIA

Experiment XXIII.

90 Madras rats
97 Belgaum rats
98 Banda rats
100 Bombay rats
} each received 1/100,000 gramme of a plague rat's spleen (=20,000 plague bacilli) on 27 May, 1912.

Date 1912	Madras			Belgaum			Banda			Bombay		
	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance	Not plague	Plague	Balance
May 27	—	—	90	—	—	97	—	—	98	—	—	100
,, 28	—	—	90	—	—	97	—	—	98	—	—	100
,, 29	—	—	90	—	—	97	—	—	98	—	—	100
,, 30	—	8	82	—	2	95	—	10	88	—	2	98
,, 31	—	33	49	—	1	94	—	32	56	—	5	93
June 1	—	20	29	—	3	91	—	19	37	—	4	89
,, 2	—	11	18	—	1	90	—	16	21	—	2	87
,, 3	—	6	12	—	2	88	—	4	17	—	4	83
,, 4	—	2	10	—	1	87	—	4	13	—	1	82
,, 5	—	2	8	—	—	87	—	2	11	—	1	81
,, 6	—	1	7	—	—	87	—	2	9	—	—	81
,, 7	—	1	6	1	—	86	—	1	8	2	—	79
,, 8	—	—	6	—	—	86	—	—	8	1	—	78
,, 9	—	2	4	—	—	86	—	—	8	—	—	78
,, 10	—	—	4	—	—	86	—	—	8	1	—	77
,, 11	—	1	3	1	—	85	—	1	7	—	1	76
,, 12	—	—	3	—	—	85	—	—	7	—	—	76
,, 13	—	—	3	—	—	85	—	—	7	—	—	76
,, 14	—	—	3	—	—	85	—	—	7	—	1	75
,, 15	—	—	3	1	—	84	—	—	7	—	—	75
,, 16	—	—	3	—	—	84	—	—	7	—	—	75
,, 17	—	—	3	—	—	84	—	—	7	—	—	75
Totals	0	87	3	3	10	84	0	91	7	4	21	75

Percentages dead of plague:

Madras	Belgaum	Banda	Bombay
97 %	10 %	93 %	21 %

NATIONAL ARCHIVES OF INDIA

As regards adult wild rats, the results are summarised in table I and in table II is given shortly the plague history of the places from which the rats were derived. Their geographical relations are shown in the appended map.

Table I shows clearly that the susceptibility to fatal plague infection varies a great deal in rats from different places, and by comparison with table II it appears that the resistance of the rats varies in a general way with the local prevalence of plague in recent years. The places investigated fall into three main groups.

(1) *Places which have suffered severely and almost continuously from plague for about ten years or more.* Bombay rats have been compared with the standard susceptible Madras rats in 18 inoculation experiments with an average mortality of 38 % against 97 % in the Madras rats. Rats from Poona have in the same way been tested against Madras and Bombay rats on 14 occasions by inoculation: the total result is that of 1390 Madras rats 1347 died (97 %), of 1387 Bombay rats 533 (38 %) and of 1355 rats from Poona only 446 (33 %). Similarly the resistance of rats from the two other large plague-infected towns of Cawnpore and Lucknow was found to be of a high grade, the summed figures of comparative experiments being

Madras, 485 out of 500 = 97 %.
Bombay, 173 out of 598 = 29 %.
Poona, 138 out of 587 = 24 %.
Cawnpore, 75 out of 414 = 18 %.
Lucknow, 117 out of 349 = 34 %.

Other towns in this group were investigated less frequently. Belgaum, where plague has for many years been exceptionally severe, yielded rats only 10 % of which died[1]. Surat (Bombay), Bellary (Madras), Bandsdeh (United Provinces) and Chit Baragaon (United Provinces) gave figures (37 to 42 % mortality) rather higher than those for Bombay (29 %) in parallel experiments, as also did Kirkee. Two other places in the United Provinces were examined—Muzaffarnagar and Delhi Shahdara—and in both places the rats were found about as little susceptible as those of Bombay. Nagpur alone of the places with a long history of severe plague gave susceptible rats, 97 and 98 % dying in two experiments. Pending further enquiries this result must be regarded as exceptional. The rats, however, were not collected

[1] In an experiment already published (vol. x. p. 458) Bombay, Poona and Belgaum rats were exposed to infected fleas: there died 18, 27 and 9 per cent. respectively.

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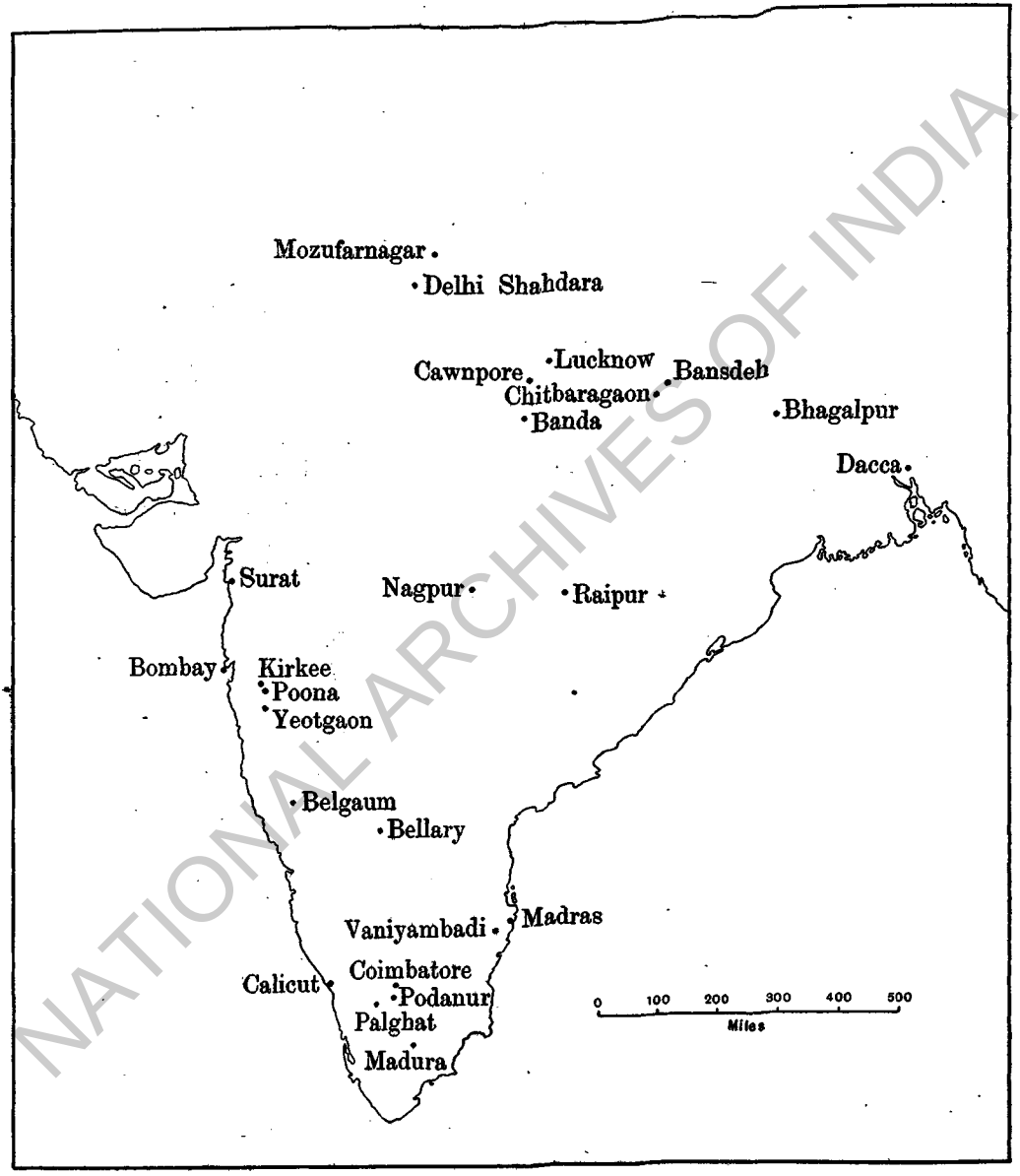

Map showing the places from which rats were obtained for immunity experiments.

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TABLE I. *Showing the percentage mortality among wild adult* Mus rattus *from various places.*

Number	Date	Dose	Madras	Bombay	Poona	Kirkee	Chit Baragaon	Bansdeh	Cawnpore	Lucknow	Bellary	Bhagalpur	Nagpur	Belgaum	Surat	Muzaffarnagar	Delhi Shaludara	Coimbatore	Calicut	Palghat	Podanur	Vaniyambadi	Raipur	Banda	Madura	Dacca	Yeotgaon	Number
I	Jan. 7, 1910	1/100,000 grm.	93	45	30	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	I
II	Jan. 28 ,,	flea infection	72	6	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	II
III	May 5 ,,	1/20,000 grm.	100	39	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	III
IV	June 2 ,,	1/20,000 ,,	95	26	10	—	—	—	—	—	—	57	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	IV
V	July 8 ,,	1/20,000 ,,	97	73	43	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	V
VI	Aug. 9 ,,	1/50,000 ,,	98	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	VI
VII	Sept. 30 ,,	1/20,000 ,,	97	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	VII
VIII	Nov. 7 ,,	1/5,000 ,,	100	73	77	95	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	VIII
IX	Jan. 5, 1911	1/5,000 ,,	100	82	78	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	IX
X	Jan. 16 ,,	1/20,000 ,,	98	26	25	40	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	X
XI	Feb. 14 ,,	1/50,000 ,,	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	XI
XII	Feb. 24 ,,	1/100,000 ,,	97	53	48	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	93	—	XII
XIII	Mar. 10 ,,	1/50,000 ,,	98	36	31	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	97	70	XIII
XIV	Apr. 22 ,,	1/100,000 ,,	99	23	—	—	38	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	XIV
XV	May 18 ,,	1/100,000 ,,	95	19	25	—	—	—	15	37	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	XV
XVI	June 21 ,,	1/100,000 ,,	100	45	—	—	—	—	20	—	—	—	—	—	—	—	—	—	—	—	—	100	—	—	100	—	—	XVI
XVII	Aug. 2 ,,	1/100,000 grm. = 26,000 bacilli	99	21	21	—	—	37	19	32	—	—	97	—	—	—	—	—	—	—	—	—	—	—	—	—	—	XVII
XVIII	Sept. 1 ,,	1/100,000 grm. = 12,000 bacilli	95	16	18	—	—	—	17	—	—	—	—	—	—	—	—	—	91	89	—	—	100	—	—	—	—	XVIII
XIX	Oct. 28 ,,	1/100,000 grm. = 20,000 bacilli	—	30	28	—	—	—	20	32	—	—	—	—	—	—	—	—	—	—	—	—	—	98	—	—	—	XIX
XX	Nov. 25 ,,	1/100,000 grm. = 18,000 bacilli	96	42	20	—	—	—	—	32	—	—	98	—	42	—	—	—	—	—	—	—	—	—	95	—	—	XX
XXI	Jan. 31, 1912	1/100,000 grm. = 16,000 bacilli	98	24	17	—	—	—	—	—	—	—	—	—	—	27	29	—	—	—	—	—	—	—	—	—	—	XXI
XXII	Apr. 27 ,,	1/100,000 grm. = 14,000 bacilli	97	27	13	—	—	—	—	—	40	—	—	—	—	—	—	60	—	—	92	—	—	—	83	—	79	XXII
XXIII	May 27 ,,	1/100,000 grm. = 20,000 bacilli	97	21	—	—	—	—	—	—	—	—	—	12	—	—	—	—	—	—	—	—	—	93	—	—	—	XXIII

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TABLE II. *Plague deaths per* 10,000.

	Population 1901	1897	1898	1899	1900	1901	1902	1903	1904	1905	1906	1907	1908	1909	1910	1911	Years infected	Average rate since commencement
Bombay[1]	776,006	136	213	202	181	208	214	269	173	191	145	88	68	68	44	55	16	149
Poona[2]	153,320	221	27	668	280	101	182	441	304	80	467	72	85	9	nil	41	13	213
Kirkee[3]	10,797	—	610	4	164	8	68	80	216	27	1	262	3	266	nil	nil	12	244
Chit Baragaon	9,505	—	—	—	—	—	425	1	197	95	394	173	18	127	173	369	10	197
Bansdeh	10,024	—	—	—	—	—	261	7	245	199	2	337	204	176	212	67	10	171
Cawnpore	183,712	—	—	—	—	—	396	282	187	103	149	62	32	47	42	106	10	141
Lucknow	264,049	—	—	—	—	—	—	160	136	206	15	234	nil	nil	nil	47	6	89
Bellary	58,247	—	—	—	—	138	241	123	167	19	1	135	1	$\frac{1}{5}$	8	66	11	82
Bhagalpur[4]	75,760	—	—	—	—	$\frac{1}{2}$	$\frac{1}{4}$	34	200	74	144	12	89	$\frac{1}{4}$	nil	nil	9	50
Nagpur[5]	127,734	—	—	—	—	—	—	755	376	7	427	12	76	571	22	150	9	274
Belgaum[6]	26,237	109	1120	814	600	392	541	418	324	132	29	33	165	145	156	93	15	338
Surat	119,306	—	160	4	6	$\frac{2}{4}$	24	244	22	341	5	43	3	nil	$\frac{1}{1\frac{1}{2}}$	$\frac{1}{3}$	13	61
Muzaffarnagar[7]	——	—	—	—	—	—	—	12	60	189	33	567	9	2	46	340	9	140
Delhi Shahdara[7]	——	—	—	—	—	—	2	24	19	76	3	151	7	$\frac{2}{5}$	22	254	10	56
Coimbatore	53,080	—	—	—	—	—	—	106	141	1	$\frac{1}{5}$	nil	nil	204	10	142	7	67
Calicut	76,981	—	—	—	—	—	—	$\frac{1}{3}$	nil	nil	nil	nil	26	10	3	7	5	5
Palghat	44,177	—	—	—	—	—	—	—	$\frac{1}{4}$	$\frac{1}{4}$	nil	nil	nil	22	7	nil	4	4
Podanur	6,568	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Vaniyambadi	12,908	—	—	—	—	594	745	1058	7	nil	nil	nil	nil	nil	nil	nil	4	219
Madras[8]	509,346	—	—	—	—	—	—	—	—	$\frac{1}{2}$	nil	nil	nil	nil	nil	nil	1	0·08
Raipur[9]	35,335	—	—	—	—	—	—	—	—	—	—	44	58	1	1	1	5	21
Banda	22,565																	
Madura	105,984																	
Dacca[10]	90,679																	
Yeotgaon	——																	

No plague

[1] Vol. VII. p. 726, vol. VIII. p. 267, this volume, p. 221. [2] Vol. VIII. p. 268, vol. X. p. 483. [3] Vol. X. p. 484.

[4] Vol. XI. supplement, p. 184. [5] Vol. VIII. p. 269. [6] Vol. VIII. p. 270, vol. X. p. 446.

[7] Details for these villages not available. The mortalities may be taken to be those of the Mozuffarnagar (see vol. X. p. 349, vol. XI. supplement, p. 106) and Meerut districts respectively, which are given above.

[8] This volume, p. 209. [9] From January to April, 1912, 425 plague deaths. [10] Vol. XI. supplement, p. 173.

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under the immediate supervision of the Commission and it may be that they came from a part of the town which has been relatively free from plague.

(2) *Places which have suffered relatively slightly from plague.* Chief among these is Madras City which has been free from plague except for one small outbreak in the outskirts of the city in 1905. The susceptibility of the rats from here is sufficiently evident. Raipur (Central Provinces) also falls in this group and has very susceptible rats. All the other five places are in the Madras presidency: Vaniyambadi suffered from two very severe epidemics some time since but has been free from plague for eight years. Calicut, Palghat and Podanur have had several small outbreaks scattered over a number of years, and the rats from all four towns proved highly susceptible. They were rather more resistant from Coimbatore, where plague prevailed rather extensively during the three years preceding the time when the rats were obtained for experiment.

(3) *Places which have been free from plague.* The four places under this heading all yielded susceptible rats. It is of some importance to note that they are widely scattered about India. The rats from Banda (United Provinces), Madura (Madras) and Dacca (Assam) were as little immune as those from Madras City. A figure was obtained indicating a rather greater resistance, though much less than in Bombay, Poona and other places in group 1, for Yeotgaon, a village in the immediate neighbourhood of Poona in which on careful enquiry we could obtain no evidence that plague had ever been present.

In comparing the figures in table I with one another it should be noted that the degrees of immunity indicated are relative to the dose, and that no very exact comparison can be made between the results of one experiment and those of another even if the dose is nominally the same since it cannot be assumed that the infectivity of the bacilli was in all cases constant. The experiments (XVII to XXIII) in which the bacilli were enumerated in a constant quantity of emulsions, made from spleens of acute plague rats showing approximately an equal number of bacilli on microscopical examination, show that $\frac{1}{100}$ mgm. contained from 12,000 to 26,000 bacilli. This is perhaps under the circumstances a very satisfactory degree of uniformity, but at the same time the range of variation shows that a dose of $\frac{1}{20}$ mgm. cannot be regarded as being necessarily greater than one of $\frac{1}{50}$ mgm. With wide differences in dosage, however, the difference in effect is plain enough: $\frac{1}{5}$ mgm. (experiments VIII and XI) killed 78 % of Bombay rats and 77 of

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those from Poona, as against 42 and 26 % with $\frac{1}{20}$ mgm., 31 and 24 % with $\frac{1}{100}$ mgm., and 6 and 2 % with flea infection[1]. It should also be understood that the dose which suitably brings out the difference between batches of rats of widely varying resistance is not necessarily an appropriate quantity for the examination of animals more nearly alike. Thus a dose of $\frac{1}{100}$ mgm. killed on the average 97 % of Madras rats, and this mortality could not be much increased under any circumstances: it rises to the limit with $\frac{1}{5}$ mgm. and falls to 72 % in the flea-infection experiment. All the rats from Vaniyambadi and Raipur were killed by the same dose of $\frac{1}{100}$ mgm. and more than 90 % from Nagpur, Calicut, Podanur, Banda, Madura and Dacca, but it does not follow that the resistance was in all cases approximately the same. Further experiments with smaller doses may show considerable variation in the rats from these different localities. In the case of Kirkee $\frac{1}{5}$ mgm. killed 95 % but a second trial with $\frac{1}{20}$ mgm. showed that the rats from this place possessed a pretty high degree of resistance. Similarly larger doses might show that the animals from Poona, Cawnpore and Belgaum really differ more widely than the present series indicates.

Taking the series of experiments as a whole it seems clear that the *resistance of rats to plague is associated with the past prevalence of plague,* and is not due to racial and local variations in susceptibility. There is of course no doubt that among a series of batches of rats all conforming to the type of *Mus rattus* it is possible to discover special and characteristic features in the specimens from certain places, not as a rule wholly confined to any one locality but being predominant in one place more than in another[2]. Thus the fur of Madras City rats is exceptionally rufous, and a very large proportion of Palghat rats had white fur on the under surface of the body while this feature was extremely rare in Poona. Rats from the colder northern places had thicker fur than southern specimens, and Belgaum rats were always on the whole larger than those caught in Bombay or Poona. But no external differences could be detected in the rats from Poona, Kirkee and Yeotgaon, which are situated within a few miles from one another. Poona has suffered severely from plague, Kirkee less severely and Yeotgaon not at all, and we found that the immunity of the rats from each place was proportionate to the degree of plague prevalence. Similarly

[1] The difficulty of conveying infection by a single flea emphasises the importance of dosage in this means of infection (see vol. VII. p. 411).

[2] See "The Races of Indian Rats," by R. E. Lloyd, in *Records of the Indian Museum,* vol. III. part 1, and especially p. 84.

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with respect to Cawnpore, Lucknow and Banda in the United Provinces. It was not possible to distinguish the rats of Cawnpore or Lucknow from those of Banda by external appearances, but on testing them by inoculation Banda rats proved to be as susceptible as Madras rats, and Cawnpore and Lucknow rats both highly immune, the former markedly more than the latter. The plague histories of the three places have been widely different; the disease has been nearly twice as severe in Cawnpore as in Lucknow, and epidemics have recurred yearly in Cawnpore while in some recent years Lucknow has escaped. Banda has been entirely free from the disease. Any local or racial variations in resistance to plague which may be associated with morphological differences such as we have indicated seem therefore to be obscured in the present series of experiments, and, as will be seen from the map, there is no evidence of any geographical distribution of immunity. Highly susceptible rats were found in such widely separated parts of India as Madras, Dacca, Madura and Banda, and highly immune animals in Bombay, Belgaum and Cawnpore. It would also be an extraordinary coincidence if plague had visited just those places where the rats were naturally most immune to the disease and spared those with the most susceptible rat populations.

It will not have escaped notice that the rats of Vaniyambadi proved to be very susceptible in 1911 and that the place had suffered from exceptionally severe plague in 1901—1903. Evidently therefore any immunity resulting from these epidemics had disappeared in seven or eight years. On the other hand we have evidence that resistance may persist during a period free from plague. In Poona the last evidence of indigenous plague was obtained in March 1909 and though the place was kept under careful observation with continuous rat examination, it remained free from plague until September 1911, a period of some 30 months. An epidemic of moderate severity then began, 630 deaths being recorded up to the end of 1911 and a further 457 by April 1912, when the outbreak came to an end. The immunity experiments cover the period from January 1910 to April 1912 and there is nothing in the results which suggests that the resistance of the rats underwent any alteration during that period. We have no definite information as to the length of life of *Mus rattus* in nature, but if the data obtained in the breeding experiments (vol. IX. supplement, p. 205) indicate with only approximate accuracy the natural rate of growth the average rat used for experimental purposes could not be more than about 6 months old. Hence in January 1910 the great majority at any rate of the rats

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tested may be presumed to have been born since the disappearance of plague from the rat population of Poona[1], and in the later experiments of 1911 there can be no doubt that this became more absolutely true in the sense that many of the animals would not only have never had a chance of experiencing epidemic plague themselves, but would be the grandchildren or great grandchildren rather than the sons and daughters of any rats which ever had. Hence we reach the conclusion that *the resistance found is not due merely to the previous reception by the experimental rats of sublethal and immunising doses of plague bacilli during the prevalence of epidemic plague but to the generation of a relatively immune progeny by the survivors of epidemics.*

This conclusion is supported by two further sets of experiments. In the first place we tested the resistance of quite young rats, from Madras, Bombay and Poona, which can have been only a few weeks old. The results show that the immunity of these baby rats is of the same

TABLE III. *Showing the percentage mortality among young wild rats.*

Experiment	Date	Dose	Madras		Bombay		Poona	
			Adult	Young	Adult	Young	Adult	Young
VI.	Aug. 9, '10	1/50 mgm.	98	—	—	47	—	39 (44*)
VII.	Sept. 30, '10	1/20 ,,	97	—	—	57 (62*)	—	20 (29*)
XI.	Feb. 14, '11	1/50 ,,	100	—	—	48 (50*)	—	29
XV.	May 18, '11	1/100 ,,	95	100	19	34	25	35
XVI.	June 21, '11	1/100 ,,	100	—	45	48	—	28

* Corrected for non-plague mortality during first three days.

order as that of the average adult rat from the same place, whether that place is and has been free from plague (Madras), is and has been heavily infected with plague (Bombay) or is free from plague with a history of severe infection in the past (Poona). Absolutely the young rats seem to be definitely rather more susceptible than the corresponding adults receiving an equal dose: this is confirmed by other experiments detailed below (p. 265).

In the second place young rats were obtained by breeding from wild rats in flea-proof godowns at Parel (vol. XI. supplement, p. 193), and as far as the results go they show that the young reproduce the resistance of their parents with remarkable fidelity.

We do not think that there is any reason to suppose that this inherited resistance is due to rats becoming immunised during an epidemic and transmitting this immunity to their offspring. The facts

[1] For some further evidence on this point, see below, p. 279 and note.

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TABLE IV. *Showing the percentage mortality among young rats bred in captivity.*

Experiment	Date	Dose	Madras		Bombay	
			Wild	Bred	Wild	Bred
III.	May 5, '10	1/20 mgm.	100	—	35	37
XVII.	Aug. 2, '11	1/100 ,,	99	—	21	20
XXII.	April 27, '12	1/100 ,,	97	100	27	22

are more easily explained by supposing that the susceptible members of a rat population are destroyed during an epidemic and that subsequent generations continue, for a time at any rate, to exhibit the resistance of the survivors. The average immunity of the rats after an epidemic will therefore in a general way be proportional to its severity, dispersion and duration, a mild epidemic leaving many rats of moderate resistance alive and a severe outbreak destroying all but those of the highest immunity. The natural process appears to be the same as that which we have already investigated in connection with the experimental godown epizootics. Thus in one series (vol. x. p. 332) 74 of 143 wild Bombay rats survived flea-borne infection in the godowns and none of them died when they were subsequently inoculated with the same dose of spleen emulsion which killed 68 of 137 (50 %) control rats. Similar results were obtained in subsequent experiments (this volume, p. 298), and the details are given above (p. 253, experiment XXII) of a test in which none of 30 survivors of experimental epidemics died with a dose which killed 97 % of Madras, 27 % of Bombay and 13 % of Poona rats. From other survivors 25 young rats were bred in captivity (p. 248, experiment XVII): of these one only died as against 99 %, 21 % and 21 % of Madras, Bombay and Poona rats respectively. If the same process went on in nature, it is easy to see how the 2 or 3 %, or with larger doses presumably the one per thousand or ten thousand, of the rat population of a place such as Madras which lived through a severe epidemic would quickly give rise to a highly resistant race[1].

Experiments on other animals.

Incidentally a few comparative experiments were also made with Madras bandicoots (experiments V and X) and with mice from Madras and Bombay (experiment I). The former show a susceptibility equal, with the doses used, to that of Madras rats (see also vol. VII. p. 760, vol. x. p. 459). Mice on the other hand proved relatively immune, whether they came from a plague-infected or a plague-free place.

[1] For the possible rate of reproduction of rats see vol. XI. supplement, p. 198.

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TABLE V. *Showing the percentage mortality from plague of bandicoots and mice compared with rats.*

Experiment	Dose	Madras rats	Bombay rats	Poona rats	Madras mice	Bombay mice	Madras bandicoots
V.	1/20,000	97	64 (73 *)	43	—	—	96
X.	1/20,000	98	26	25	—	—	96
I.	1/100,000	83 (93 *)	44	30	58 (71 *)	54 (63 *)	—

* Corrected for early mortality from causes other than plague: see p. 232, above.

SUMMARY.

1. *Rats from different places show varying degrees of immunity to plague.*

2. *This immunity is relative to the infecting dose.*

3. *Immunity is greatest in places which have suffered most severely from plague, less marked in those places which have suffered to a moderate extent and least in those places in which epidemic plague has not occurred.*

4. *This immunity is not always acquired from an attack of plague, and is transmitted by the parents to their offspring which have not been exposed to infection.*

APPENDIX.

On the influence of size and sex on the percentage mortality.

Experiments such as those detailed above have involved the use of rats varying somewhat in size; quite small animals were, however, never used except in the particular experiments designed to test the resistance of young rats. It is important therefore to know whether a moderate variation in size or any sexual differences in the various batches of rats would be likely to lead to any considerable variation in the results of inoculation, the same dose being used whatever the size of the animal. The pretty regular uniformity in the results for Madras and Bombay rats with which many experiments were made is evidence that these factors have not introduced any very material error. We give here, however, the analysis of another series of observations made in Poona in 1909 with the idea of testing for seasonal changes in resistance (see vol. x. p. 523). All the animals received as far as possible the same dose of spleen emulsion. Table VI gives the results for 2000 consecutive rats (77 of which died from causes other than plague) in groups of 500 classified by weight, and table VII deals with the same animals grouped also by sex.

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TABLE VI.

Weight grammes	Jan. 22 to Feb. 28	March 2 to April 13	April 13 to May 18	May 19 to June 25	Total inoculated	Total died of plague	Per cent. died of plague
11—30	15/45 = 33 %	25/61 = 41 %	43/84 = 51 %	39/80 = 49 %	270	122	45·2
31—50	23/67 = 34	38/128 = 30	15/54 = 28	38/82 = 46	331	114	34·4
51—70	11/58 = 19	12/48 = 25	28/78 = 36	28/83 = 34	267	79	29·6
71—90	32/65 = 49	15/53 = 28	22/70 = 31	17/67 = 25	255	86	33·7
91—110	43/112 = 38	19/88 = 22	25/92 = 27	23/65 = 35	357	110	30·8
111—130	23/57 = 40	11/49 = 22	10/52 = 19	18/59 = 31	217	62	28·6
131—150	15/41 = 37	6/38 = 16	11/42 = 26	13/41 = 32	162	45	27·8
151—170	5/15 = 33	7/13 = 54	1/9 = 11	3/11 = 27	48	16	33·3
171—210	2/4 = 50	0/4 = 0	0/4 = 0	2/4 = 50	16	4	25·0
Totals	169/464 = 36 %	133/482 = 28 %	155/485 = 32 %	181/492 = 37 %	1923	638	33·2 %

It appears from this that young rats are definitely more susceptible than older individuals[1], the dose being the same for all weights. The difference is however in no way proportional to the weight and is small relatively to the differences dealt with above in rats from different

TABLE VII.

Weight grammes	Males			Females		
	Inoculated	Died	Per cent.	Inoculated	Died	Per cent.
71— 90	109	38	35	146	48	33
91—110	141	50	35	216	60	28
111—130	89	29	32	128	33	26
131—150	88	28	32	74	17	23
151—170	30	12	40	18	4	22
171—210	13	3	23	3	1	33
Totals	470	160	= 34·0 %	585	163	= 27·9 %

places. Among grown-up rats there is no definite relation between weight and percentage mortality. As regards sex, table VII shows that females are apparently somewhat less resistant than males, but the difference is not sufficient to enable one to definitely exclude the errors of chance selection.

[1] At the time these experiments were made, some of the older animals had probably had experience of a plague epidemic.

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LII. CHRONIC OR RESOLVING PLAGUE.

In previous numbers of these reports (vol. VI. p. 580, vol. VII. pp. 457, 719, vol. X. p. 335) some remarks have been made and observations recorded on the condition called chronic plague, or, as we prefer to name it, resolving plague. In order to obtain a more complete knowledge of this condition, we decided to make a careful examination daily of some two hundred rats (100 *decumanus* and 100 *rattus*) caught alive in Bombay city. We proposed to carry on this examination for a whole year. At the same time we continued to gain experience of this condition in other places in India, for example at Poona, and we were able to compare the frequency and character of the lesions we found in rats in these plague-infected centres with similar lesions found in rats in the plague-free city of Madras.

I. *Resolving plague in Bombay.*

The first part of this paper deals with our observations in Bombay city. In our early experience of plague we failed to observe or at least to note the lesions we now recognise to be often produced by the plague bacillus and which we have called resolving plague lesions. This was not to be wondered at for several reasons, among the most important of which was the fact that these resolving lesions have very little resemblance to the lesions found in the acute stages of the disease; they were therefore passed over as due to some unknown cause. Moreover, in our early examination of rats in Bombay, the work was so organised that, while the animals were cut up by one individual, a detailed bacteriological examination was made some hours later by another. In the meantime the surface of the abscesses and other lesions became contaminated with organisms which in cultures inhibited or obscured the growth of the plague bacillus[1]. We also relied almost altogether on the cutaneous method of infection in guinea-pigs when we subjected any

[1] Reference should be made here to the single case of chronic plague which was noted in our early observations in Bombay: the case was detected by isolating the plague bacillus from a mixed culture by the use of a special medium (vol. VII. p. 468).

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suspected material to animal tests. We now know that this method of infection is not often successful in demonstrating the presence of the comparatively small number of living plague bacilli found in these resolving plague lesions. But as our experience enlarged, especially when we came to deal with an increased number of rats which had been trapped alive and not found dead (as the majority were which we examined in Bombay in the early part of our observations), the relation of the resolving plague lesions to the acute lesions gradually unfolded itself. With the fuller knowledge gained by this extended experience we decided to re-examine the Bombay rats for evidence of resolving plague.

In our last paper (vol. x. p. 340) we endeavoured to lay down certain rules to enable us to distinguish and classify acute plague lesions from resolving lesions. We recognised that the rules were arbitrary and that a series of plague-infected rats might be selected which showed almost imperceptible gradations from the characteristic acute lesions to the very different resolving lesions. We hoped, however, for convenience of classification and comparison, by aid of these rules to divide plague-infected rats into three groups, viz. those with (*a*) acute lesions, (*b*) resolving lesions, (*c*) resolved lesions. Experience during the observations now about to be detailed, however, showed that the distinction we endeavoured to make between acute plague and the early stages of resolving plague was too fine to be practical and another group was therefore added to the series, viz. rats with appearances intermediate between the typical acute lesions and the well-marked and localised lesions of resolving plague. The term "subacute plague" was used to designate this group of rats. Our difficulties in classification, however, did not end here for, since no arbitrary line of distinction could be drawn between the rats of the different groups and as the observations extended over periods of more than a year, a uniform classification became almost impossible. A careful perusal of the notes, which were compiled from day to day, leaves little room for doubt that the grouping of the rats at the end of the observations was not just exactly the same as at their commencement, and this was particularly the case with the rats classed in the groups acute, subacute and resolving plague. For convenience of record, however, some classification was necessary in order to deal conveniently with the large amount of material we had collected. Little harm will result from errors in classification if this explanation serves to impress on the reader a fact which was patent to the workers, that the lesions we have attempted to classify under

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separate heads formed in reality a continuous series of pathological changes produced, for the most part by the plague bacillus, from acute, through healing, to completely healed lesions.

Table I shows the number of rats examined each month in Bombay from September 1909 to August 1910, *decumanus* and *rattus* separately. The figures show the number found with (*a*) acute plague, (*b*) subacute plague, (*c*) resolving plague, (*d*) resolved, recovered or post-plague lesions. Table II gives a more detailed classification of the resolving and resolved lesions. It is hardly necessary to explain that many rats showed more than one of the lesions represented in the classification adopted in tables I and II, but each rat was recorded under one head only. A rat for example which showed perisplenitis, a scar in the spleen and adhesions between the spleen and the abdominal wall was classed under the head adhesions. Again, a rat which showed an abscess in the spleen with scars, perisplenitis and adhesions was recorded under the heading abscess of the spleen, that is under the heading which, in our opinion, represented the earliest stages in the process of resolution.

Before analysing further the material we have collected and before describing a few typical examples of the lesions recorded under each head, it remains only to remark that the observations in Bombay extended from the 16th August, 1909, to the 19th September, 1910. The rats had always been trapped alive and to all appearance were healthy before they were killed. As far as possible 100 *decumanus* and 100 *rattus* were examined daily except on Sundays and on certain other holidays. In the early part of the observations it was not possible to collect 100 rats of each species, but later the full number was obtained daily. In the case of rats suffering from acute or subacute plague no note was made of the species found infected, but in the case of rats suffering from resolving or resolved lesions complete notes were kept. It is interesting to observe that *M. decumanus* showed rather more than twice as many individuals with lesions under these heads as *M. rattus*. We have shown elsewhere (vol. VII. p. 750) that *M. decumanus* suffers rather more than twice as severely from acute plague as does *M. rattus*. This is probably explained by the fact that the average number of fleas found on *M. decumanus* in Bombay is rather more than twice as large as on *M. rattus* (see also vol. VIII. p. 297). Since all the rats we examined in the present series were trapped alive and since *decumanus* suffered rather more than twice as severely from resolving and resolved plague lesions than *rattus* did, the facts are in favour of the difference in

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TABLE I.

Month	Number of rats examined			No. of acute plague rats	Per cent. of acute plague rats	No. of subacute plague rats	Per cent. of subacute plague rats	Resolving plague				Resolved plague			
	decumanus	*rattus*	Total					No. of *decumanus*	Per cent. of *decumanus*	No. of *rattus*	Per cent. of *rattus*	No. of *decumanus*	Per cent. of *decumanus*	No. of *rattus*	Per cent. of *rattus*
Sept. ...	2057	2089	4146	1	·02	0	0	12	·58	8	·38	21	1·02	11	·52
Oct. ...	2454	2500	4954	0	0	0	0	17	·69	3	·12	20	·81	12	·48
Nov. ...	2088	2200	4288	0	0	1	·02	21	1·06	6	·27	24	1·15	8	·36
Dec. ...	2000	2000	4000	0	0	0	0	15	·75	6	·30	31	1·15	12	·6
Jan. ...	2300	2300	4600	0	0	8	·17	14	·6	2	·08	50	2·19	11	·47
Feb. ...	2400	2400	4800	0	0	35	·73	16	·66	4	·16	36	1·5	33	1·37
March ...	2100	2100	4200	1	·02	35	·83	10	·48	5	·24	26	1·23	24	1·14
April ...	2500	2500	5000	4	·08	48	·96	16	·64	3	·12	32	1·28	21	·84
May ...	2500	2500	5000	10	·2	41	·82	4	·16	1	·04	34	1·36	11	·44
June ...	2400	2400	4800	2	·04	25	·52	10	·42	1	·04	48	2·00	25	1·04
July ...	2500	2500	5000	12	·24	5	·1	12	·48	2	·08	42	1·69	23	·92
Aug. ...	2400	2400	4800	6	·12	2	·04	12	·5	2	·08	43	1·8	26	1·08
Totals ...	27699	27889	55588	36	·06	200	·36	159	·57	43	·15	407	1·47	217	·77

TABLE II.

Month	No. of rats examined			Resolving plague: Chronic buboes		Abscesses of spleen		Necrotic areas in spleen		Post-plague lesions: Adhesions		Scars		Bisected spleen		Perisplenitis	
	decumanus	*rattus*	Total	*decumanus*	*rattus*	*decumanus*	*rattus*	*decumanus*	*rattus*	*decumanus*	*rattus*	*decumanus*	*rattus*	*decumanus*	*rattus*	*decumanus*	*rattus*
Sept. ...	2057	2089	4146	8	3	3	3	1	2	10	11	3	0	3	0	5	0
Oct. ...	2454	2500	4954	10	0	0	1	5	2	12	10	1	0	4	1	3	1
Nov. ...	2088	2200	4288	12	5	6	0	5	1	12	6	3	0	5	0	4	2
Dec. ...	2000	2000	4000	9	3	1	0	5	3	13	8	10	0	8	1	0	3
Jan. ...	2300	2300	4600	6	1	4	0	4	1	21	8	12	1	8	0	9	2
Feb. ...	2400	2400	4800	6	1	5	2	5	1	17	24	10	6	5	1	4	2
March ...	2100	2100	4200	2	1	0	0	8	4	18	20	1	3	4	1	3	0
April ...	2500	2500	5000	6	1	5	2	5	0	22	18	4	0	5	1	1	2
May ...	2500	2500	5000	0	0	0	0	4	1	18	11	5	0	8	0	3	0
June ...	2400	2400	4800	2	0	4	1	4	0	30	23	6	0	8	1	4	1
July ...	2500	2500	5000	2	0	4	2	6	0	24	19	6	3	6	1	6	0
Aug. ...	2400	2400	4800	4	0	6	0	2	2	26	19	9	5	6	1	2	1
Totals ...	27699	27889	55588	67	15	38	11	54	17	223	177	70	18	70	8	44	14

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the number of fleas being the explanation of the observed difference in severity of the disease in the two species rather than the alternative explanation, which was at the same time advanced, that, owing to its habits, *decumanus* was more likely to be found dead by the collecting staff than *rattus*.

The lesions described under the heading "resolving plague" are, we believe, of comparatively recent development, that is to say they have not been present in the rat for many months. They are generally the result of an attack of plague from which the rat is recovering. These lesions always afford some morbid material which is capable of being submitted to bacteriological examination. It is not, however, always possible to prove that these lesions are due to the plague bacillus either because it is sometimes difficult to obtain an uncontaminated culture from them, or because the lesions have become sterile or secondarily infected by some other organism than the plague bacillus. It must be clearly understood, however, that while we believe that the majority of these lesions were originally produced by the plague bacillus, even although it is not always possible to demonstrate the plague bacillus in them, we feel sure that similar lesions to those produced by the plague bacillus are brought about by some other cause or causes. Evidence to this effect will be forthcoming when we describe our findings in the rats caught in Madras city.

A. *Resolving plague.*

We classify resolving lesions into three groups: (1) chronic buboes, (2) necrotic areas in the spleen, (3) chronic abscesses in the spleen and more rarely in the liver.

(*a*) *Chronic buboes.*

Discussing first chronic buboes, a detailed analysis has been made of 78 rats presenting these lesions. In two of this number the condition was associated with necrotic areas in the spleen and in two cases with resolved lesions in the spleen. Of the buboes 68 were cervical, nine were inguinal and one was axillary[1]. The buboes were generally single, and varied in size from a small pea to a haricot bean, the smaller ones being the commoner. Generally they were firm and hard, with thick gristly walls, and when incised were found to contain

[1] *I.e.* the distribution was the same as in acute plague rats (vol. VII. pp. 329, 386, 388, 826, 829, 910, vol. X. p. 469).

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a small bead of pus. The pus was, in some cases, of a pale yellow colour and creamy in consistence, but in other cases the material contained in the buboes was dry and inspissated. *B. pestis*, when found microscopically, was present in those glands containing fluid pus, not in those containing inspissated material. The buboes were usually firmly fixed to the surrounding tissues, but there was no local congestion, oedema or haemorrhage surrounding them.

Of the 78 rats examined, the contents of the buboes of seven when injected subcutaneously into guinea-pigs produced plague in these animals. Five of these buboes were cervical, one inguinal and one axillary.

In two cases	the guinea-pig	died in	three days.
In three cases	„	„	six days.
In one case	„	„	seven days.
In one case	„	„	nineteen days.

In seven cases a pure culture of *B. pestis* was obtained while material from the buboes failed to kill guinea-pigs. In 27 cases cultures from the buboes proved to be sterile. In 14 cases plague-like bacilli were seen microscopically in smears made from the buboes, and in five of these cases the cultures were nevertheless sterile and in nine others a growth was obtained which was not the plague bacillus. In the remaining 23 cases no evidence of plague was obtained by microscopical, cultural or animal tests, and cultures gave a growth other than plague.

It will thus be seen that about 9 % of the buboes contained a sufficient number of plague bacilli sufficiently virulent to kill guinea-pigs when the pus from the buboes was injected subcutaneously into them. Further 9 % of the buboes contained plague bacilli which were isolated by culture while the contents of the buboes failed to kill guinea-pigs. In 18 % of the cases organisms which resembled the plague bacilli were seen microscopically in smears, but that they were really plague bacilli was not proved by culture or animal experiment. In 64 % of the cases no definite evidence was obtained to show that the lesions were produced by the plague bacillus.

(*b*) *Necrotic areas in the spleen.*

An analysis has been made of 74 rats which showed necrotic ar in the spleen; two of these cases, as has been remarked above, associated with chronic buboes. The necrotic conditions in the were of four types. (1) Small nodules, about the size of a m

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or a lentil, situated as a rule just beneath the capsule of the spleen. They are well defined and of a firm consistence and are simply rather larger and more clearly defined than the necrotic spots found in the spleen and liver in acute plague (vol. VII. p. 332). The necrotic spots, as they enlarge and pus forms within them, give rise to the abscesses in the spleen described below. (2) Wedge-shaped necrotic areas were met with; their shape and appearance suggested that they had been produced by infarction. The base of the wedge was usually at the upper or lower margin of the spleen and the apex extended for a varying distance across the organ, often reaching half-way through its substance. These areas were of a pale yellow colour tinged with pink or of a more distinctly pink colour. In some cases the outline of the organ was preserved uninterruptedly over the necrotic area, but in other cases, especially those in which organisation and cicatrisation had commenced, an indentation of the border was produced which, as healing progressed, remained as a scar on the surface of the spleen. Rats showing scars of this kind have been classed among those with resolved or post-plague lesions, *i.e.* when all the necrosed tissues had been absorbed. (3) Band-shaped necrotic areas extended right across the spleen, generally completely through its substance. They varied in breadth from a narrow strip to a broad band, and in colour and appearance resembled the wedge-shaped necrotic areas described above. In some cases these bands bulged out from the surface of the spleen, in other cases they did not alter the contour of the organ. In those cases in which the healing process had advanced, and in which cicatrisation had taken place, the spleen became constricted at the site of the lesion. Complete organisation of these lesions led to the formation of a transverse scar, a condition we have classified as bisected spleen among the post-plague lesions. In some cases these band-shaped necrotic areas were multiple, and we have found transverse scars in the spleen associated with other bands which had not completely cicatrised, and in some of these cases we were able to demonstrate the presence of plague bacilli in the necrosed tissue which remained. (4) Large irregular necrotic areas were infrequent, but they sometimes involved as much as two-thirds to three-quarters of the entire area of the spleen, only small portions of healthy tissue being left. When such lesions had healed portions of the spleen were sometimes left widely separated from one another but connected together by strands of fibrous tissue. Of the 74 rats with [redacted]tic areas in the spleen, 11 were shown to harbour living plague [redacted] by injecting an emulsion of the necrosed tissue subcutaneously [redacted]ea-pigs.

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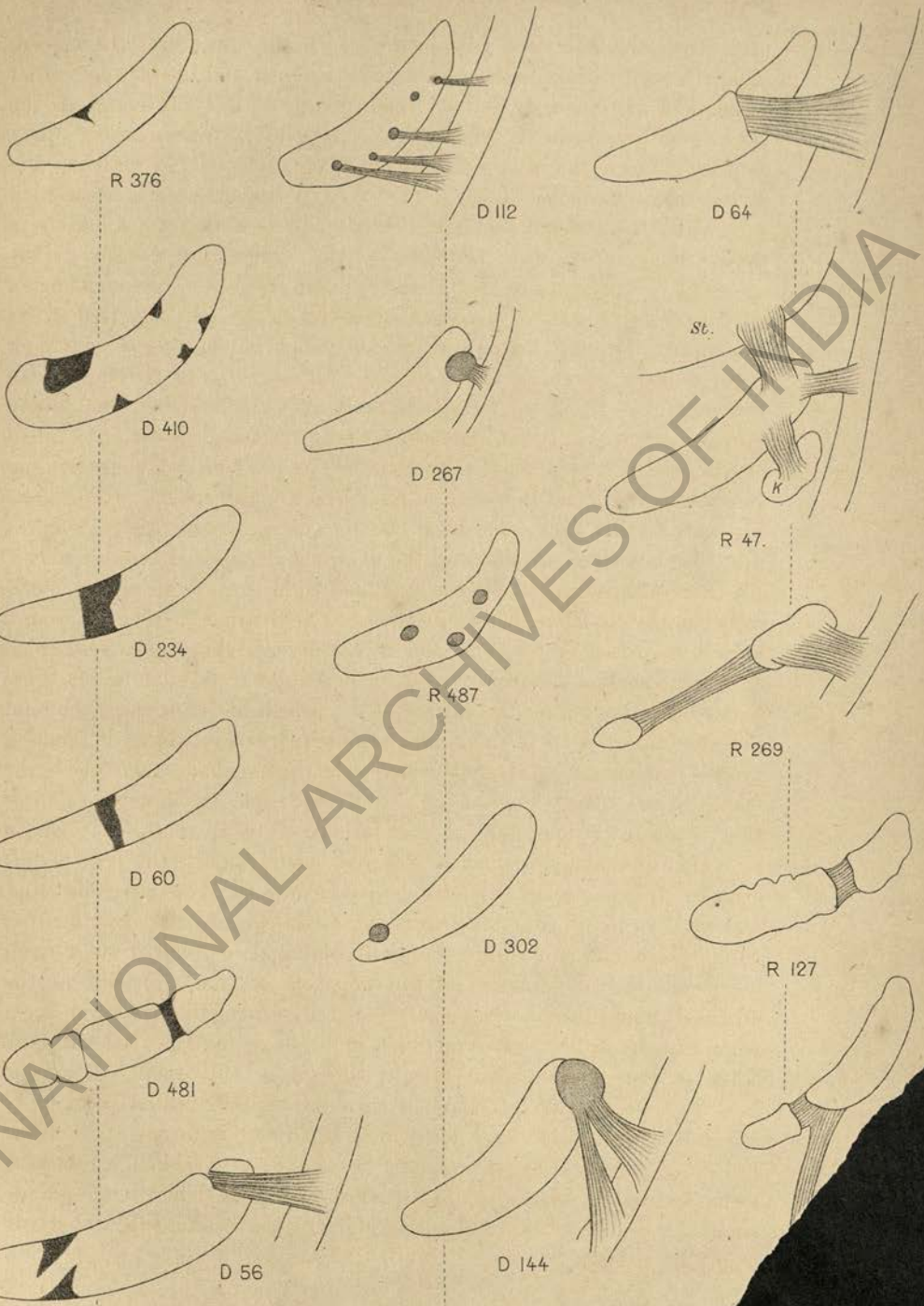

Necrotic areas in spleen Inoculation positive with 376, 234, 481 and 56.

Abscesses in spleen Inoculation positive with 112, 487, 302 and 144.

Recov... adh... wa... Al...

DIAGRAMS OF RESOLVING PLAGUE LES...

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One guinea-pig	died of plague	on the	second	day	after injection.
One	„	„	third	„	„
Three guinea-pigs	„	„	fourth	„	„
One guinea-pig	„	„	sixth	„	„
Two guinea-pigs	„	„	ninth	„	„
One guinea-pig	„	„	eleventh	„	„

Two more were chloroformed on the 19th and 26th days after injection and were found to be recovering from an attack of plague. In two cases, in which the material which had been injected into guinea-pigs had failed to produce plague in them, the plague bacillus was isolated from the lesions in pure culture. Twenty-four cases proved to be sterile. Three showed plague-like organisms microscopically, but other tests designed to detect the presence of the bacillus failed. In 34 cases growths other than plague were obtained, and other tests for plague were negative. It thus appears that about 15 % of the necrotic areas examined contained the *B. pestis* as demonstrated by the guinea-pig test. In 3 % of the cases *B. pestis* was isolated in cultures although the animal test failed. In 4 % bacilli resembling plague bacilli were seen microscopically, although this finding was not confirmed by other tests. 32 % of the cases proved to be sterile. In 78 % no direct evidence of plague was obtained.

(*c*) *Abscesses in the spleen or liver.*

Passing on to consider abscesses in the spleen we found that these varied in size from a small pea to an almond but a few large abscesses containing as much as three drachms of pus were met with. The abscesses were generally single but occasionally a few to many were found in the spleen of one rat. The walls of the abscesses were often thick and fibrous, and definitely marked off from the surrounding splenic tissue. In 25 instances the abscesses were fixed to adjoining structures by bands of adhesion. These adhesions most often extended between the abscess and the abdominal wall, and next in frequency between the abscess and the mesentery. In a few cases the abscesses were adherent to the stomach, intestines, liver, or kidney. The abscesses usually contained thick yellowish white pus. In some cases the pus was inspissated and dry. Bacteriological examination of 36 of the abscesses gave the following result. The contents of the abscess injected subcutaneously into guinea-pigs produced plague in these animals in 15 instances. In two cases, while the material injected into guinea-pigs failed to kill the animals, a pure culture of *B. pestis* was obtained from the

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lesions. In five cases plague-like bacilli were seen in smears, but no other proof of their existence in the abscess was obtained. In four cases the contents of the abscesses were sterile. In fourteen (39 %) rats no evidence of the presence of the plague bacillus was obtained, and the cultures in these cases yielded other organisms than the plague bacillus. The results of the examination of these splenic abscesses shows that 42 % of them contained sufficiently numerous or sufficiently virulent plague bacilli to cause the development of the disease in guinea-pigs.

The series of cases shows (*a*) that some cases in which proof of the presence of plague bacilli was obtained resembled very closely others in which no evidence of the presence of these bacilli was forthcoming. (*b*) They show the relation which exists between this type of lesion and those described under the term resolved plague. In the latter cases the healing process has progressed a stage further; the plague bacilli have been destroyed, pus and necrotic tissue have been absorbed, adhesions, scars and perisplenitis remain the sole evidence of previous infection. There is no longer any material from which living plague bacilli can be isolated.

B. *Resolved or post-plague lesions.*

The conditions which we have to describe under this head are almost wholly connected with the spleen. They may be classified as follows:

(1) Adhesions of the spleen to surrounding structures.
(2) Scars in the spleen.
(3) Bisected or trisected spleen.
(4) Fibrous thickening of the capsule of the spleen, "perisplenitis."

(1) *Adhesions* were the most common of the four conditions. They were found between the spleen and surrounding structures in the following order of frequency:

(1)	To the abdominal wall	301	cases.
(2)	To the omentum	110	„
(3)	To the left kidney	35	„
(4)	To the stomach	23	„
(5)	To the uterus and ovary	17	„
(6)	To the vesiculae seminales	7	„
(7)	To the intestines	7	„
(8)	To the liver	4	„
(9)	To the bladder	1	case.

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Adhesions of the abdominal wall and the omentum were often present together and an adhesion to one of the abdominal organs was often associated with adhesion to the abdominal wall.

The adhesions in some cases consisted of a firm matting of the spleen to the adjacent structures so that the organ was rendered quite immoveable. In other cases fibrous bands stretched between the spleen and adjoining structures, only partially fixing the spleen. These bands were of various degrees of thickness and strength. Multiple adhesions were frequently present, they almost always arose from some scar on the surface of the spleen or from patches of perisplenitis.

(2) *Scars* were present, singly or combined with other lesions, especially with adhesions. The commonest situation for scars was near one or other border of the spleen, and there the margin of the spleen was indented. These scars and indentations were frequently multiple and in some cases caused considerable distortion of the spleen. Some scars extended transversely across the spleen, generally completely through the whole thickness of the organ. Occasionally small depressed scars were met with on the surface of the spleen where apparently necrotic spots had once existed.

(3) *Bisected spleens*, as the term indicates, were divided into two portions, the two parts being separated by a definite interval filled in by scar tissue. The separation of the two parts varied in degree, being as much as one inch in one case. Trisection was noted in a few cases. Adhesions frequently extended from the bisecting scar to the abdominal wall or to one of the abdominal organs.

(4) *Perisplenitis* generally existed as a well-defined area of pearly-grey colour. These areas varied much in size, in some cases they covered the greater portion of the surface of the spleen, but in other cases the areas were more numerous but smaller. Perisplenitis was often associated with scars in the spleen.

An analysis of some cases in which resolving lesions were associated with resolved lesions.

Resolving plague lesions as has been stated above were frequently associated with resolved lesions. The following are details of some of the combinations found:

Splenic abscess with	adhesion to the abdominal wall	...	10 cases.
„	„ adhesion to the omentum	...	4 „
„	„ adhesions to the kidney	...	2 „

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Splenic abscess with		adhesion to the stomach	1 case.
„	„	adhesion to the intestines	2 cases.
„	„	adhesions to the abdominal wall, stomach and liver	2 „
„	„	adhesion to the abdominal wall and stomach	2 „
Necrotic spots	„	adhesion to the abdominal wall... ...	5 „
„	„	adhesion to the abdominal wall, bisected spleen and scar	1 case.
Necrotic spot	„	adhesion to the abdominal wall and uterus	1 „
Necrotic spots	„	adhesions to the omentum	5 cases.
Necrotic spot	„	scar	1 case.
„	„	perisplenitis	1 „

II. *Observations in Poona.*

We may pass on now to record our experience of resolving and resolved plague in Poona city. Our observations in this place extended from June 1908 to March 1912. During this period 141,287 rats were examined. All these rats were trapped alive in the city of Poona and were all *Mus rattus*. The observations here are particularly interesting, since they covered two epidemics of the disease, one at the commencement and one at the close of the observations with a practically plague-free interval of nearly two and a half years between the two epidemics. During this interval very careful inquiries showed that the disease was neither epidemic nor epizootic in the city save only on two occasions when some evidence was collected to prove that limited epizootics were present, started probably by the importation of infection from outside the city. The one epizootic occurred in a part of the city called Nana's Peth early in November 1909, and the other in Shanwar Peth in March 1911. All the evidence goes to show that neither of these epizootics extended beyond the rats living in one or two houses in the immediate vicinity of one another.

What has been said above in regard to difficulties in classification of the lesions found in Bombay rats applies with equal force to the investigations in Poona. Here the early observations from June to December 1908 are unreliable as to the number of rats found with resolving and resolved plague lesions. These early observations were largely instrumental, as has been explained, in unfolding to us the relation of these

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TABLE III.

Table showing the number and percentage of acute plague, resolving plague (visceral and peripheral lesion) and recovered plague (adhesions only) met with in Poona Mus rattus *caught alive in regular trapping since June* 1908.

Month		Total live *rattus* examined	Acute plague alive		Resolving plague		Recovered plague (only adhesions)	
			Number	Monthly percentage	Number	Monthly percentage	Number	Monthly percentage
June	1908	3977	—	—	3	0·08	—	—
July	,,	5351	—	—	4	0·07	—	—
August	,,	5946	—	—	4	0·07	—	—
September	,,	6115	10	0·16	18	0·29	—	—
October	,,	4913	29	0·59	27	0·55	—	—
November	,,	3814	30	0·78	29	0·76	3	0·08
*December	,,	2923	27	0·92	77	2·63	26	0·88
January	1909	2376	6	0·25	33	1·39	43	1·81
February	,,	2284	1	0·04	22	0·97	57	2·50
March	,,	2906	—	—	18	0·62	48	1·65
April	,,	2644	—	—	5	0·19	28	1·06
May	,,	2479	—	—	5	0·20	19	0·77
June	,,	2830	—	—	4	0·14	22	0.78
July	,,	3727	—	—	7	0·19	31	0·83
August	,,	3554	—	—	6	0·17	24	0·68
September	,,	3520	—	—	13	0·37	25	0·71
October	,,	3508	—	—	5	0·14	21	0·60
November	,,	4059	—	—	8	0·20	21	0·52
December	,,	4306	—	—	7	0·16	15	0·35
January	1910	3462	—	—	7	0·20	12	0·35
February	,,	3659	—	—	3	0·08	6	0·16
March	,,	3273	—	—	4	0·12	7	0·21
April	,,	3120	—	—	1	0·03	3	0·10
May	,,	2875	—	—	3	0·10	2	0·07
June	,,	2916	—	—	7	0·24	7	0·24
July	,,	3140	—	—	10	0·32	8	0·25
August	,,	3534	—	—	17	0·48	7	0·20
September	,,	2712	—	—	4	0·15	7	0·26
October	,,	3628	—	—	11	0·30	9	0·25
November	,,	4408	—	—	22	0·50	8	0·18
December	,,	4298	—	—	12	0·28	9	0·21
January	1911	3380	—	—	4	0·12	11	0·33
February	,,	2929	—	—	3	0·10	13	0·44
March	,,	3290	—	—	3	0·09	17	0·50
April	,,	2775	—	—	5	0·18	13	0·47
May	,,	2622	—	—	2	0·08	5	0·19
June	,,	2211	—	—	2	0·09	6	0·27
July	,,	2703	—	—	0	—	11	0·41
August	,,	2631	—	—	0	—	6	0·23
September	,,	2765	1	0·04	5	0·18	1	0·04
October	,,	1840	8	0·43	13	0·71	10	0·54
November	,,	2401	12	0·50	28	1·16	11	0·46
December	,,	1953	9	0·46	45	2·30	27	1·38
January	1912	1802	3	0·17	49	2·72	21	1·17
February	,,	1527	3	0·20	21	1·38	33	2·16
March	,,	1201	—	—	10	0·83	28	2·33
Total		148287	139	0·09	586	0·40	681	0·46

* Owing to difficulties in classification figures for recovered plague lesions prior to December 1908 are not reliable.

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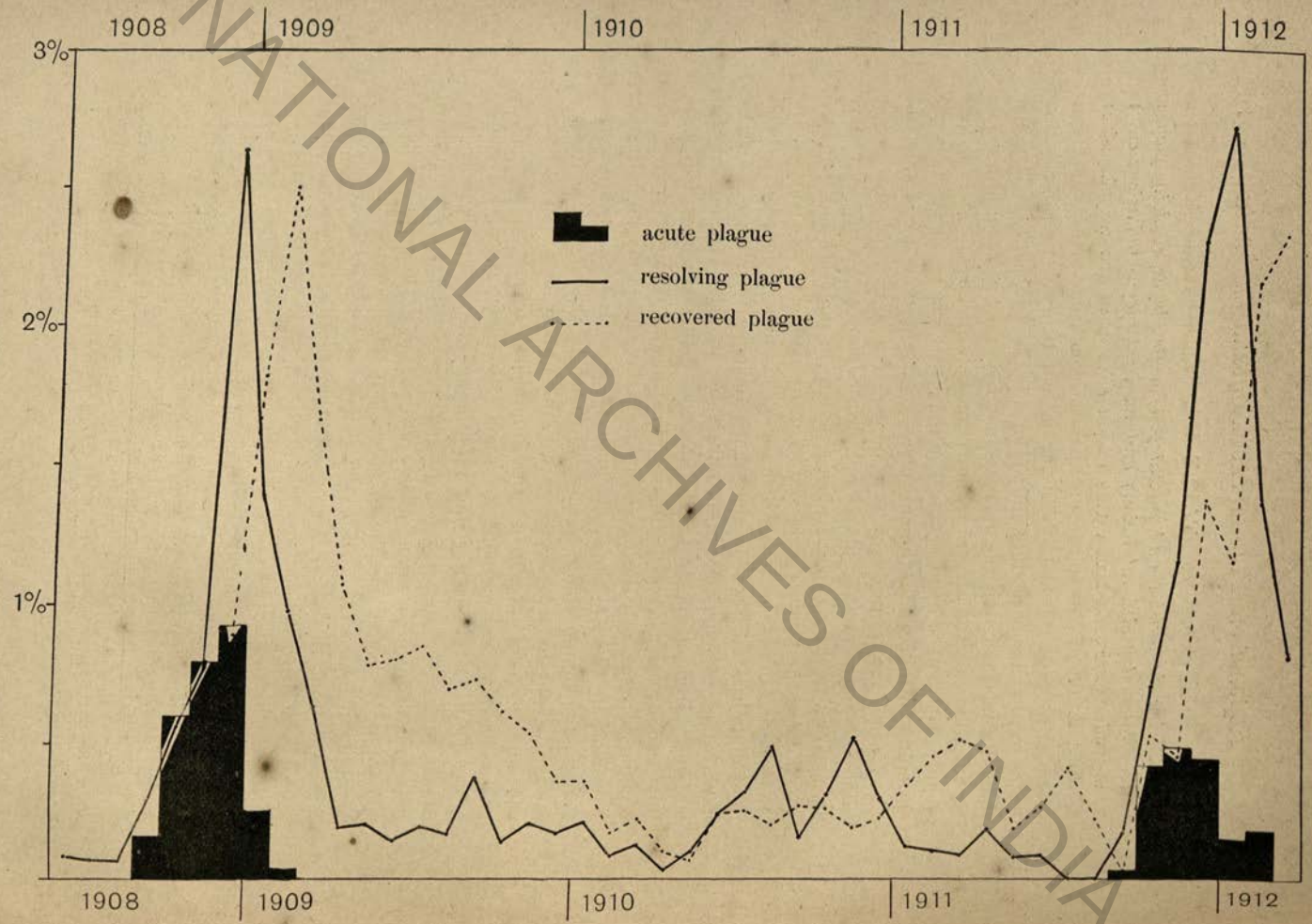

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lesions to the plague bacillus. The rats which have been classed as sub-acute plague, in our Bombay observations, have been distributed in Poona between the acute and resolving groups. The criteria as to what constituted a scar in the spleen or what degree of thickening of the capsule of the spleen entitled the condition to be called perisplenitis differed considerably in Poona and Bombay, the observations being in the hands of different workers in the two places. The same difficulty in classifying the rats was not however experienced in regard to adhesions of the spleen to surrounding structures. Here a very definite fact had to be ascertained so that there was no variation in the grouping in this case in the two places or in the same place when the observations were made over a period of years. For this reason adhesions between the spleen and surrounding structures only have been shown in the group of resolving lesions in table III and the chart, which detail the number of rats examined each month in Poona for the whole period of observations, which extended to nearly four years. This table and chart clearly show that the pathological conditions which we have called resolving and resolved plague, in spite of the fact that we were often unable to obtain absolute proof that they had been produced by the plague bacillus by isolating that organism from them, occur most frequently during and immediately after a plague epizootic[1]. Nevertheless there is ample evidence to show that sometimes lesions very similar in appearance to those produced by the plague bacillus are brought about by some cause as yet unknown to us for these lesions continue to occur, it is true with diminished frequency, even when plague has not been epizootic for years. Moreover, as will be seen in tables IV to VIII, even young rats showed these lesions, and they could not have been exposed to plague infection for they must have been born at a time when no plague was present in the city. Table IX shows how far it was possible to prove that the lesions we have called resolving plague were due to the plague bacillus by isolating that organism from them. In this table the so-called resolving plague lesions have been divided into two groups, visceral and peripheral; the former includes abscesses and necrotic areas in the spleen and liver while in the latter are grouped all buboes and abscesses in the lymphatic glands. It will be observed that only a small number (17 %) of resolving

[1] These observations may throw important light on the length of life of *rattus* in nature. Assuming that adhesions do not tend to shorten the animal's life and, which is more problematical, that they do not disappear, it is clear that the absence of any excess of such lesions about a year after a plague epidemic involves the death within that period of the bulk of the rat population alive at the time of the epidemic.

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lesions could be shown to contain living plague bacilli while, from the very nature of the lesions, it was not possible to demonstrate the presence of plague bacilli in the resolved lesions. Attention has to be drawn to two confirmed rats with visceral lesions in June and August 1909. From the age of the rats, as judged by their weight, the character of the lesions and the circumstances in which the rats were found we have reason to think that these had been infected some time towards the close of the first epizootic, which, as far as we could obtain evidence,

TABLE IV.

Incidence of glandular lesions (abscesses as well as necrotic areas) according to the age of the rat in quarterly periods from the commencement of observations in Poona.

Quarter ending		Young *rattus*			Adult *rattus*			Total *rattus* (adult and young)		
		Rattus examined	No. with glandular lesions	Percentage	*Rattus* examined	No. with glandular lesions	Percentage	*Rattus* examined	No. with glandular lesions	Percentage
December	1908*	2963	8	0·27	3799	43	1·13	6762	51	0·75
March	1909	3673	3	0·08	3914	41	1·05	7587	44	0·58
June	,,	4478	1	0·02	3485	6	0·17	7963	7	0·09
September	,,	5638	3	0·05	5193	13	0·25	10831	16	0·15
December	,,	5443	5	0·09	6446	12	0·19	11889	17	0·14
March	1910	4336	1	0·02	6266	9	0·10	10602	10	0·09
June	,,	4041	3	0·07	4886	5	0·10	8927	8	0·09
September	,,	4152	2	0·05	5234	10	0·19	9386	12	0·13
December	,,	5999	2	0·04	6335	17	0·27	12334	19	0·15,
March	1911	3704	2	0·05	5895	5	0·08	9599	7	0·07
June	,,	3599	2	0·06	4009	4	0·10	7608	6	0·08
September	,,	3936	0	—	4163	2	0·05	8099	2	0·02
December	,,	2589	6	0·23	3605	32	0·89	6194	38	0·61
March	1912	1582	4	0·25	2948	38	1·29	4530	42	0·93
Totals		56133	42	0·07	66178	237	0·36	122311	279	0·23

* Figures for November and December only, those prior to November not available and figures for November not reliable.

came to an end about the month of March, so that these rats had harboured plague bacilli in their bodies for from three to five months. Both rats had abscesses in the spleen. Attention has also to be drawn to three rats caught in November 1909 which showed cervical buboes from which the plague bacillus was isolated. These three rats were associated with one of the limited epizootics which we mentioned occurred during the plague-free interval. The affected rats were young rats, not three months old, so that they must have acquired infection

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TABLE V.

Incidence of splenic lesions (abscesses as well as necrotic areas) according to the age of the rat in quarterly periods from the commencement of observation in Poona.

Quarter ending		Young *rattus* less than 70 grammes			Adult *rattus* 70 grammes and over			Total *rattus* (adult and young)		
		Rattus examined	No. with splenic abscesses	Percentage	*Rattus* examined	No. with splenic abscesses	Percentage	*Rattus* examined	No with splenic abscesses	Percentage
December	1908*	2963	13 (*a*)	0·44	3799	42 (*b*)	1·11	6762	55	0·81
March	1909	3673	2	0·05	3914	26 (*c*)	0·66	7587	28	0·37
June	,,	4478	1	0·02	3485	6	0·17	7963	7	0·09
September	,,	5638	3	0·05	5193	6	0·12	10,831	9	0·08
December	,,	5443	0	—	6446	2	0·03	1889	2	0·02
March	1910	4336	0	—	6266	3	0·05	10,602	3	0·03
June	,,	4041	1	0·02	4886	2	0·04	8927	3	0·03
September	,,	4152	7	0·17	5234	12	0·23	9386	19	0·20
December	,,	5999	9	0·15	6335	17	0·27	12,334	26	0·21
March	1911	3704	0	—	5895	3	0·05	9599	3	0·03
June	,,	3599	1	0·03	4009	2	0·05	7608	3	0·04
September	,,	3936	3	0·08	4163	0	—	8099	3	0·04
December	,,	2589	19 (*d*)	0·73	3605	29 (*e*)	0·80	6194	48	0·77
March	1912	1582	7 (*f*)	0·44	2948	31 (*g*)	1·05	4530	38	0·84
Totals.....		56,133	66	0·12	66,178	181	0·27	122,311	247	0·20

* Figures for November and December only, those prior to November not available and figures for November not reliable.

(*a*) One *rattus* with combined visceral and peripheral lesions included amongst visceral and not amongst peripheral.

(*b*) Eight *rattus* with combined visceral and peripheral lesions included amongst visceral and not amongst peripheral types.

(*c*) Four *rattus* with combined visceral and peripheral lesions included amongst visceral and not amongst peripheral types.

(*d*) Four *rattus* with combined visceral and peripheral lesions included amongst visceral and not peripheral.

(*e*) Ten *rattus* with combined visceral and peripheral lesions included amongst visceral and not peripheral.

(*f*) Three *rattus* with combined visceral and peripheral lesions included amongst visceral and not peripheral.

(*g*) Thirteen *rattus* with combined visceral and peripheral lesions included amongst visceral and not peripheral.

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at some date after March 1909, when we believe the first epizootic terminated. Excepting the rats mentioned above, none were found harbouring living plague bacilli during the plague-free interval, although a most careful examination by animal, cultural and microscopical tests was made of every suspected rat. Despite this fact, however, we have

TABLE VI.

Splenic scars in quarterly periods since commencement of observations in Poona. Young and adult rattus shown separately.

Quarter ending		Young *rattus*			Adult *rattus*			Total *rattus* (adult and young)		
		Rattus examined	No. with scars	Percentage	*Rattus* examined	No. with scars	Percentage	*Rattus* examined	No. with scars	Percentage
December	1908*	2963	3	0·10	3799	22	0·58	6762	25	0·37
March	1909	3673	14	0·38	3914	53	1·35	7587	67	0·88
June	,,	4478	9	0·20	3485	60	1·72	7963	69	0·87
September	,,	5638	38	0·67	5193	89	1·71	10831	127	1·17
December	,,	5443	36	0·66	6446	118	1·83	11889	154	1·30
March	1910	4336	8	0·18	6266	90	1·44	10602	98	0·92
June	,,	4041	25	0·62	4886	67	1·37	8927	92	1·03
September	,,	4152	36	0·87	5234	133	2·54	9386	169	1·80
December	,,	5999	66	1·10	6335	200	3·16	12334	266	2·16
March	1911	3704	42	1·13	5895	116	1·97	9599	156	1·65
June	,,	3599	28	0·78	4009	83	2·07	7608	111	1·46
September	,,	3936	52	1·32	4163	109	2·62	8099	161	1·99
December	,,	2589	47	1·82	3605	123	3·41	6194	170	2·74
March	,,	1582	7	0·44	2948	78	2·65	4530	85	1·88
Totals		56133	411	0·73	66178	1341	2·03	122311	1750	1·43

* Figures for November and December only, those prior to November not available and figures for November not reliable.

little doubt that many of the lesions we found were primarily produced by the plague bacillus. It cannot be denied, however, as has been frequently stated above, that some of the lesions were produced by other unknown cause or causes so that the classification "resolving" and "resolved" plague is to that extent misleading.

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TABLE VII.

Perisplenitis in quarterly periods since the commencement of observations in Poona.

Quarter ending		Young *rattus*			Adult *rattus*			Total *rattus* (adult and young)		
		Rattus examined	No. with perisplenitis	Percentage	*Rattus* examined	No. with perisplenitis	Percentage	*Rattus* examined	No. with perisplenitis	Percentage
December	1908*	2963	5	0·17	3799	35	0·92	6762	40	0·59
March	1909	3673	10	0·27	3914	101	2·58	7587	111	1·46
June	,,	4478	5	0·11	3485	42	1·20	7963	47	0·59
September	,,	5638	5	0·09	5193	38	0·73	10,831	43	0·40
December	,,	5443	0	—	6446	31	0·48	11,889	31	0·26
March	1910	4336	0	—	6266	34	0·54	10,602	34	0·32
June	,,	4041	0	—	4886	25	0·51	8927	25	0·28
September	,,	4152	5	0·12	5234	41	0·78	9386	46	0·49
December	,,	5999	1	0·02	6335	33	0·52	12,334	34	0·28
March	1911	3704	1	0·03	5895	39	0·66	9599	40	0·42
June	,,	3599	1	0·03	4009	25	0·62	7608	26	0·34
September	,,	3936	6	0·15	4163	37	0·89	8099	43	0·53
December	,,	2589	12	0·46	3605	33	0·92	6194	45	0·73
March	1912	1582	2	0·13	2948	42	1·42	4530	44	0·97
Totals ...	...	56,133	53	0·09	66,178	556	0·84	122,311	609	0·50

* Figures for November and December only, those prior to November not available and figures for November not reliable.

TABLE VIII.

Adhesions of spleen in quarterly periods since commencement of observations in Poona.

Quarter ending		Young *rattus*			Adult *rattus*			Total *rattus* (adult and young)		
		Rattus examined	No. with adhesions	Percentage	*Rattus* examined	No. with adhesions	Percentage	*Rattus* examined	No. with adhesions	Percentage
December	1908*	2963	1	0·03	3799	28	0·74	6762	29	0·43
March	1909	3673	9	0·25	3914	138	3·53	7587	147	1·94
June	,,	4478	0	—	3485	69	1·98	7963	69	0·87
September	,,	5638	6	0·11	5193	74	1·42	10831	80	0·74
December	,,	5443	6	0·11	6446	51	0·79	11889	57	0·48
March	1910	4336	2	0·04	6266	23	0·37	10602	25	0·24
June	,,	4041	0	—	4886	12	0·24	8927	12	0·13
September	,,	4152	1	0·02	5234	21	0·40	9386	22	0·23
December	,,	5999	6	0·10	6335	20	0·32	12334	26	0·21
March	1911	3704	2	0·05	5895	39	0·66	9599	41	0·43
June	,,	3599	2	0·06	4009	22	0·55	7608	24	0·32
September	,,	3936	1	0·03	4163	17	0·41	8099	18	0·22
December	,,	2589	7	0·27	3605	41	1·14	6194	48	0·77
January	1912	1582	2	0·13	2948	80	2·71	4530	82	1·81
		56133	45	0·08	66178	635	0·96	122311	680	0·56

* Figures for November and December only, those prior to November not available and figures for November not reliable.

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TABLE IX.

Table showing the sensational prevalence of plague and so-called post plague lesions amongst rattus *of regular (Poona City) trapping in monthly periods from June* 1908 *to March* 1912.

Months		Total alive *rattus* examined	Acute plague		Resolving plague				Recovered plague		
					Visceral		Peripheral				
			Dead	Alive	Confirmed	Not confirmed	Confirmed	Not confirmed	Adhesions	Scars	Perisplenitis
June	1908	3977	—	—	—	2	—	1	—	—	—
July	,,	5351	—	—	—	—	—	4	—	—	—
August	,,	5946	—	—	—	1	—	3	—	—	—
September	,,	6115	24	10	2	—	2	14	—	—	—
October	,,	4913	36	29	3	14	3	7	—	—	—
*November	,,	3814	5	30	5	14	3	7	3	3	3
December	,,	2923	1	27	3	34	7	33	26	22	37
January	1909	2376	2	6	1	13	4	15	43	24	55
February	,,	2284	1	1	2	8	2	10	57	17	30
March	,,	2906	—	—	—	5	—	13	48	26	22
April	,,	2644	—	—	—	4	—	1	28	12	14
May	,,	2479	—	—	—	2	—	3	19	25	15
June	,,	2830	—	—	1†	—	—	3	22	32	18
July	,,	3727	—	—	—	2	—	5	31	42	19
August	,,	3554	—	—	1‡	3	—	2	24	31	11
September	,,	3520	—	—	—	4	—	9	25	54	13
October	,,	3508	—	—	—	1	—	4	21	33	3
November	,,	4059	—	—	—	1	3§	4	21	58	10
December	,,	4306	—	—	—	1	—	6	15	63	18
January	1910	3462	—	—	—	1	—	6	12	37	8
February	,,	3659	—	—	—	2	—	1	6	35	10
March	,,	3273	—	—	—	1	—	3	7	26	16
April	,,	3120	—	—	—	—	—	1	3	29	7
May	,,	2875	—	—	—	1	—	2	2	22	8
June	,,	2916	—	—	—	2	—	5	7	41	10
July	,,	3140	—	—	—	4	—	6	8	52	14
August	,,	3534	—	—	—	13	—	4	7	61	17
September	,,	2712	—	—	—	2	—	2	7	56	15
October	,,	3628	—	—	—	8	—	3	9	85	11
November	,,	4408	—	—	—	14	—	8	8	92	9
December	,,	4298	—	—	—	4	—	8	9	89	14
January	1911	3380	—	—	—	1	—	3	11	56	14
February	,,	2929	—	—	—	1	—	2	13	56	13
March	,,	3290	—	—	—	1	—	2	17	46	13
April	,,	2775	—	—	—	2	—	3	13	37	11
May	,,	2622	—	—	—	—	—	2	5	41	8
June	,,	2211	—	—	—	1	—	1	6	33	7
July	,,	2703	—	—	—	—	—	—	11	42	13
August	,,	2631	—	—	—	—	—	—	6	61	13
September	,,	2765	1	8	—	3	—	2	1	58‖	17
October	,,	1840	8	15	1	8	—	4	10	48¶	10
November	,,	2401	12	12	5	12	2	9	11	64	18
December	,,	1953	9	4	6	16	12	11	27	58	17
January	1912	1802	3	5	8	14	15	12	21	38	18
February	,,	1527	3	0	2	7	6	6	33	24	20
March	,,	1201	—	—	2	5	1	2	28	23	6

* Figures for post plague lesions not reliable before November 1908.

† This rat was caught alive at Raviwar 174 on June 7, 1909 (wt. 130 grms.).

‡ This rat was caught alive at Bhowanee 1004 on Aug. 19, 1909 (wt. 170 grms.).

§ (1) Caught alive at 179 Nana on Nov. 3, 1909 (wt. 85 grms.). (2) Caught alive at 186 Nana on Nov. 13, 1909 (wt. 52 grms.). (3) Caught alive at 186 Nana on Nov. 13, 1909 (wt. 80 grms.).

‖ Three of these were either scars or perisplenitis.

¶ One of these was either scar or perisplenitis.

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III. *The examination of Madras rats.*

We propose now to support this latter contention by detailing the result of the examination of 15,523 rats caught alive in Madras city, which has been free from plague since 1905[1]. Table X shows for each month during the year 1910 the number of rats examined and those found with lesions which might have been classed under the terms "resolving" or "resolved" plague as defined above but which could not have been produced by the plague bacillus. In no case was any organism resembling the plague bacillus found. In all lesions were found in 0·23 % of the rats examined.

TABLE X.

Month	Number of rats examined	Number with visceral lesions	Number with peripheral lesions
January	3151	2	0
February	1978	2	0
March	1197	2	1
April	1011	4	0
May	1502	2	1
June	1331	1	1
July	1007	3	2
August	899	0	2
September	965	3	0
October	1132	4	1
November	1021	3	0
December	329	1	0
Totals	15523	27	8

List of Madras rats with lesions resembling resolving and resolved plague lesions.

	1910	
5551	Jan. 10	Perisplenitis with adhesions
5726	,, 11	Perisplenitis with adhesions to omentum.
8079	Feb. 5	Single chronic abscess in the spleen.
8914	,, 12	Perisplenitis.
9616	Mar. 3	Left kidney atrophied to half size adherent to spleen.
9770	,, 5	Submaxillary abscess with inspissated pus; not plague.
10646	,, 30	Perisplenitis; spleen adherent to kidney.
10789	April 2	Perisplenitis; spleen adherent to abdominal wall.
11134	,, 12	Solitary chronic abscess of spleen; no evidence of plague.
11191	,, 23	Spleen constricted and adherent to abdominal wall.
11175	,, 23	Several necrotic areas in spleen; adherent abdominal wall.
13010	May 28	Left inguinal abscess; no evidence of plague.
13026	,, 28	Adhesions between spleen and abdominal wall.
13347	,, 7	Liver and spleen adherent to the stomach.
14303	June 23	Submaxillary abscess; no evidence of plague.
14486	,, 25	Liver, spleen and intestines matted together by strong fibrous adhesions; spleen adherent to abdominal wall, a chronic abscess in liver with thick yellow pus.

[1] See above, p. 209.

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14672	July 5	Spleen, liver and intestines matted together; spleen adherent to abdominal wall; abscesses in liver; not plague.
14811	,, 11	Right inguinal abscess; not plague.
14842	,, 12	Spleen adherent to abdominal wall.
15009	,, 19	Spleen adherent to abdominal wall.
15095	,, 21	Right axillary abscess with thick cheesy pus; not plague.
15828	Aug. 12	Submaxillary abscess; not plague.
15939	,, 15	Submaxillary abscess with cheesy pus.
16454	Sept. 1	Perisplenitis, spleen adherent to abdominal wall.
16900	,, 17	Spleen adherent to abdominal wall and kidney.
17092	,, 23	Bisected spleen.
17700	Oct. 14	A small area of perisplenitis.
17767	,, 15	Spleen adherent to abdominal wall.
18013	,, 19	Left submaxillary abscess.
18149	,, 21	Spleen adherent to abdominal wall and kidney.
18360	,, 26	Adhesion between spleen and abdominal wall.
18763	Nov. 5	Liver and spleen adherent to the stomach.
19223	,, 19	Spleen adherent to the stomach and the abdominal wall.
19297	,, 21	Spleen adherent to the stomach and abdominal wall.
19898	Dec. 9	Lower portion of the spleen replaced by fibrous tissue and adherent to the kidney.

Conclusions.

1. Lesions, for which we propose the terms resolving and resolved plague lesions, are found in rats which have been subjected to plague infection and in certain individuals are stages in the natural process of recovery from the acute form of this disease.

2. These lesions are very different from the lesions found in the acute stages of the disease. They are generally localised and confined for the most part to the spleen.

3. It is not always possible to prove that these lesions are due to the plague bacillus by isolating that bacillus from them, for in many cases the bacilli have been killed, have disappeared or been replaced by some other organism.

4. That the lesions are frequently produced by the plague bacillus has been shown by (*a*) demonstrating the presence of plague bacilli in some of these lesions which exactly resemble others, either in the same rat or in other rats, in which no plague bacilli can be found, (*b*) by showing that the lesions increase in numbers during and after epizootics of the disease.

5. Nevertheless the fact must be borne in mind that very similar lesions can be produced by other causes, as has been shown by our experience of the examination of rats in Madras city, where the disease is not present.

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LIII. THE EXPERIMENTAL PRODUCTION OF RESOLVING PLAGUE AND POST-PLAGUE LESIONS IN RATS.

I. In the course of some experiments on the relative immunity to plague of rats from different parts of India, the details of which are given above (p. 229), a large number of rats were inoculated with varying small doses of plague bacilli. The doses given were quite small and a considerable number of the rats inoculated survived. Of these survivors a certain percentage showed lesions similar to what we have described (above, p. 266) as resolving plague and post-plague lesions in naturally infected rats. In all, 3600 rats were inoculated and of these 1079 survived. The survivors were killed by chloroform three weeks after inoculation and examined. 119 or 11 % of these showed resolving plague or post-plague lesions.

Table I shows the number of rats from each source, the numbers and percentages of survivors and of survivors showing resolving and post-plague lesions.

It will be seen that, excluding the Bhagalpur and Yeotgaon figures which are based on a single experiment, the rats showing the greatest susceptibility to plague also show the largest number of resolving plague lesions among the survivors. That is, in addition to the rats which have died of plague a considerable number of the remainder have passed through a moderately severe attack which has left well-marked signs behind it. Thus of 1000 Madras rats inoculated, 9 of the 18 survivors showed resolving lesions, only 9 rats showing no sign of having passed through an attack of plague. The converse also holds true, the rats which are very resistant to plague showing only a small percentage of resolving lesions among the survivors. For example, out of 300 young Poona rats, 160 survived and 5 only of these showed resolving plague lesions, the remaining 155 showing no sign of having passed through an attack.

Wild rats such as those used in these experiments are of course liable to have chronic plague when inoculated. In the preceding paper the data are given on this point as regards Madras, Bombay and Poona. 1000 Madras rats would probably include 2 rats with post-plague lesions, and an average sample of 700 Bombay or Poona rats

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about 6 or 8 animals with resolving and recovered plague. Even if all these survived the test inoculations, they will not account for the considerable number actually found.

TABLE I.

	Number inoculated	Number of survivors	Percentage of survivors	Number of survivors with resolving and post-plague lesions	Percentage of survivors with resolving and post-plague lesions
Madras rats	1000	18	2	9	50
Dacca rats	200	10	5	3	30
Kirkee rats	200	61	30	19	31
Adult Bombay *rattus* ...	700	287	41	24	8
Young Bombay *rattus* ...	300	127	42	5	4
Adult Poona rats ...	700	344	49	46	13
Young Poona rats ...	300	160	53	5	3
Bhagalpur rats ...	100	43	43	7	16
Yeotgaon rats	100	29	29	1	3

The following lesions were found :—

Lesion	Number of cases
Pelvic buboes	28
Abscesses in spleen	55
,, liver	6
,, kidney	1
Necrotic areas in spleen	10
Adhesions of spleen to abdominal wall	30
,, ,, omentum	9
,, ,, kidney	3
,, ,, stomach	1
,, ,, ovary	1
Scars in spleen	11
Perisplenitis	17

The lesions were single in 83 cases and multiple in 36, two or more lesions, such as abscesses in the spleen and adhesion to the abdominal wall, being associated in many cases.

Resolving Plague Lesions.

Buboes:—28 cases. These were pelvic in situation, the injection being always given in the groin. They were in all respects similar to the chronic buboes found in wild rats.

Abscesses in the spleen:—55 cases. These varied in number from 1 to 30 or 40 and in size from a pinhead to a small pea. 1 larger abscess about the size of a cherry was found associated with 2 of the typical small abscesses.

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Adhesions between the surfaces of the abscesses and the surrounding structures were present in 26 cases as under:—

Adhesion to abdominal wall	16
,, omentum	3
,, omentum and abdominal wall ...	3
,, kidney	1
,, kidney and abdominal wall ...	2
,, abdominal wall and ovary ...	1

Scars in the spleen and perisplenitis were associated with abscesses in a few cases.

Abscesses in Liver:—6 cases. In 4 cases there were also abscesses in the spleen and adhesions of the spleen to surrounding structures. The liver abscesses resembled those in the spleen in appearance.

Necrotic areas in the spleen:—10 cases. In 5 out of the 10 cases these areas were small spots of well-defined appearance, well marked off from the surrounding tissues, and unassociated with other lesions. In one case necrotic spots of this sort were present along with abscesses in the spleen, and in another along with adhesion to the abdominal wall. In the remaining 3 cases the necrotic areas were of the "band" or wedge type and were associated with other lesions as follows:—

1. Necrotic band + adhesion to omentum.
2. Necrotic band + necrotic wedge + scar + adhesion to abdominal wall and stomach.
3. Necrotic band + adhesion to abdominal wall and omentum + abscesses in the spleen.

Post-Plague Lesions.

Adhesions between the spleen and neighbouring structures:—37 cases. The adhesions were with a single structure in 30 cases and with more than one structure in 7 cases. The order of frequency was the same as in naturally infected rats, adhesion with the abdominal wall being commonest and adhesion with the omentum next in frequency.

The following were the lesions in the spleen found associated with adhesions:—

	Cases
Abscesses in spleen	25
Abscesses and necrotic areas in spleen	1
Necrotic areas in spleen	4
Perisplenitis	3
Scars	2
No other lesion	2
Total	37

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It will be seen that in the great majority of cases (about 95 %) the adhesions were associated with conditions which were obviously due to a recent acute attack of plague. The adhesions, when associated with abscesses, always commenced directly from the surface of the abscesses and were short and friable and of recent formation. When associated with necrotic areas they were as a rule in the form of matting rather than definite bands. When associated with perisplenitis or scars only or when no other lesion was associated, the adhesions were longer and firmer and formed well-organised tough bands.

Perisplenitis:—17 cases. This was usually in the form of small well-defined surface patches of a glistening pearly white appearance. In several cases it was associated with adhesions of the spleen to surrounding structures.

All these lesions exactly resemble those we have found in naturally infected rats and the circumstances of their occurrence show clearly that they are stages on the way to recovery from an attack of plague of moderate severity.

II. During the attempt to measure any seasonal variation in immunity of the rats at Poona which was made during 1908–9 (vol. x. p. 523, and *supra*, p. 229), in all 3800 rats were inoculated in the right thigh with a small dose of spleen emulsion. Of these 371 died of causes other than plague, 1321 died of acute plague and 2108 survived for 21 days when they were killed with chloroform and examined. At that time our views on resolving plague were undeveloped. We have, however, a record of the occurrence of encapsuled abscesses and some other details which are of interest. Abscesses containing plague-like organisms, the contents of which gave plague to experimental animals in the great majority of cases in which the test was used, were found as follows, the lesions in 31 cases being multiple.

	Right	Left	Both	Total
Submaxillary and cervical glands ...	7	6	5	18
Axillary glands	6	3	0	9
Pelvic glands	227	2	3	232
Inguinal glands	33	2	0	35
Total glandular abscesses	...	...	...	294
Liver	...	...	...	8
Spleen	...	...	...	18

Altogether 296 animals out of 2108 (14·1 %) had abscesses; visceral abscesses were noted in only 26 cases (1·2 %). As has been shown

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above (p. 276), such abscesses would be expected in about 5 per thousand of wild Poona *rattus* or about 20 in the original 3800 animals. Evidently, therefore, the lesions were mostly produced by the experimental inoculation.

The distribution of the buboes in the 1321 rats dying of acute plague may be compared with the distribution of the glandular abscesses: the two series give very similar results:—

	Acute plague buboes	Chronic abscesses
Submaxillary ...	1·3 %	4·5 %
Axillary	0·4	1·4
Pelvic	79·7	74·9
Inguinal	3·4	8·5
Multiple	15·2	10·7
Total	100·0	100·0

To give some idea of the time relations of these processes we give also the proportion of the inoculated rats dying at various periods after inoculation:

1st day	nil	
2nd	12 per thousand	
3rd	285	
4th	287	
5th	156	
6th	103	
7th	69	1st week 912 per thousand
8th	35	2nd week 74 ,,
9th	11	3rd week 14 ,,
10th	6	
11th	11	
12th	5	
13th	4	
14th	2	
15th to 21st days ...	14	

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LIV. EXPERIMENTAL PLAGUE EPIDEMICS AMONG RATS.

(Fourth Communication.)

In the last report (vol. x. p. 315) the details of five experimental epizootics with *Mus rattus* were given. The data of three more are now available and these eight experiments together form a series extending over a complete year from September 1909.

The experiments may be summarised as follows:—

Experiment I. Sept. 24th to Nov. 2nd, 1909.

Godown	Inoculated rats: Put in	Inoculated rats: Dead of plague	Uninoculated rats: Put in	Uninoculated rats: Dead of plague	Plague rats eaten	Flea count
No. 7	10	9	15	1	0	1 found on Sept. 27 contained *B. pestis* in its stomach
No. 8	10	9	15	0	0	Nil
No. 9	10	8	15	0	0	Nil
No. 10	10	10	15	2	0	Average 2·5 per rat
No. 11	10	8	15	3	0	Average 1·4 per rat
No. 12	10	8	15	0	1	Average 7 per rat

Experiment II. Nov. 5th to Dec. 13th, 1909.

Godown	Inoculated rats: Put in	Inoculated rats: Dead of plague	Uninoculated rats: Put in	Uninoculated rats: Dead of plague	Plague rats eaten	Flea count
No. 7	14	10	15	0	0	One flea found on Nov. 15
No. 8	15	13	15	0	1	One flea found on Nov. 29
No. 9	14	12	15	0	0	Nil
No. 10	10	10	15	5	0	Average 13 per rat
No. 11	10	9	15	5	4	Average 18 per rat
No. 12	10	10	15	7	0	Average 39 per rat

Experiment III. Dec. 21st, 1909, to Jan. 24th, 1910.

Godown	Inoculated rats: Put in	Inoculated rats: Dead of plague	Uninoculated rats: Put in	Uninoculated rats: Dead of plague	Plague rats eaten	Flea count
No. 7	5	5	15	0	0	Godown flea infested on Jan. 17
No. 8	5	5	15	0	1	Nil
No. 9	5	4	15	0	2	Nil
No. 10	5	5	15	10	1	Average 34 per rat
No. 11	5	5	15	6	0	Average 29 per rat
No. 12	5	5	15	11	0	Average 48 per rat

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Experiment IV. Feb. 3rd to March 10th, 1910.

Godown	Inoculated rats: Put in	Inoculated rats: Dead of plague	Uninoculated rats: Put in	Uninoculated rats: Dead of plague	Plague rats eaten	Flea count
No. 7	10	8	15	0	0	Nil
No. 8	10	9	15	0	0	One flea found on March 7
No. 9	10	8	15	0	0	Two fleas found on March 7
No. 10	10	7	15	9	0	Average 20 per rat
No. 11	10	8	15	6	1	Average 14 per rat
No. 12	10	9	15	4	0	Average 15 per rat

Experiment V. March 22nd to May 2nd, 1910.

Godown	Inoculated rats: Put in	Inoculated rats: Dead of plague	Uninoculated rats: Put in	Uninoculated rats: Dead of plague	Plague rats eaten	Flea count
No. 7	15	15	15	0	0	One flea found on April 25
No. 8	15	14	15	0	0	Nil
No. 9	15	14	15	0	0	Four fleas found on April 25
No. 10	5	5	15	15	0	Average 11 per rat
No. 11	15	15	15	12	0	Average 30 per rat
No. 12	15	13	15	1	0	Average 21 per rat

Experiment VI. May 10th to June 13th, 1910.

Godown	Inoculated rats: Put in	Inoculated rats: Dead of plague	Uninoculated rats: Put in	Uninoculated rats: Dead of plague	Plague rats eaten	Flea count
No. 7	5	5	15	0	0	Nil
No. 8	5	5	15	0	0	Nil
No. 9	5	4	15	0	0	Nil
No. 10	5	5	15	6	1	Average 10·3 per rat
No. 11	5	5	15	14	0	Average 15·3 per rat
No. 12	5	5	15	10	0	Average 16 per rat

Experiment VII. June 25th to August 1st, 1910.

Godown	Inoculated rats: Put in	Inoculated rats: Dead of plague	Uninoculated rats: Put in	Uninoculated rats: Dead of plague	Plague rats eaten	Flea count
No. 7	10	6	15	0	0	Nil
No. 8	10	8	15	0	0	Two fleas found on August 1
No. 9	10	8	15	0	0	Nil
No. 10	5	5	15	3	2	Average 14 per rat
No. 11	5	5	15	10	0	Average 20·7 per rat
No. 12	5	5	15	11	0	Average 32 per rat

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Experiment VIII. August 15th to September 26th, 1910.

Godown	Inoculated rats: Put in	Inoculated rats: Dead of plague	Uninoculated rats: Put in	Uninoculated rats: Dead of plague	Plague rats eaten	Flea count
No. 7	10	8	15	0	0	Nil
No. 8	10	8	15	0	0	Nil
No. 9	10	6	15	0	0	Nil
No. 10	10	9	15	3	0	Average 14 per rat
No. 11	10	8	15	6	1	Average 18 per rat
No. 12	10	9	15	7	0	Average 17 per rat

The whole series of eight experiments are shortly summed up in the following table:

TABLE I.

Number	Date, 1909—10	Control godowns (7, 8, 9): Rats died=per cent.	Control godowns (7, 8, 9): Fleas	Flea godowns (10, 11, 12): Rats died=per cent.	Flea godowns (10, 11, 12): Fleas per rat
I	24th Sept.—2nd Nov.	1/45 = 2 %	1 found	5/45 = 11 %	4
II	5th Nov. —13th Dec.	0/45	2 found	17/45 = 38	23
III	21st Dec. —24th Jan.	0/45	present at end	27/45 = 60	37
IV	3rd Feb. —10th Mar.	0/45	3 found	19/45 = 42	16
V	22nd Mar.— 2nd May	0/45	none	28/45 = 62	21
VI	10th May —13th June	0/45	none	30/45 = 67	14
VII	25th June — 1st Aug.	0/45	2 found	24/45 = 53	22
VIII	15th Aug. —26th Sept.	0/45	none	16/45 = 36	16
Totals		1/360=0·3 %		166/360=46 %	

These experiments fully confirm those already published as to the predominant influence of the presence of fleas on the spread of plague infection from the infected rats put into the godowns to their healthy companions. In the present communication we desire to discuss the variations in the severity of the epizootics in the flea-infected godowns in the different experiments.

1. *Flea prevalence.* The flea population in the godowns was produced by the introduction of fleas removed from wild rats, a hundred or so being put into each godown every few days throughout the first epizootic and during the first half of the second experiment. Most of the fleas introduced in this way appear to die very soon, possibly on account of the handling involved and exposure to the sun during the process of collection, and it is some time before breeding is well established and a strong flea population available. It will be seen that there were relatively few fleas present during the first experiment, an increase during the second and a large rise during the third. Thereafter the flea prevalence fell somewhat and remained fairly constant. The fleas in the godowns were to some extent subject to

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unnatural interference from the removal of infected rats, the presence of only a small number of rats in the intervals between the epizootics and the partial flooding of the floors of the godowns during the rainy months (June, July).

The data show that, with the exception of the fifth and sixth experiments, there is a very fair relationship between the flea prevalence and the number of rats dying from plague. This is especially well shown in the correspondence between the increasing severity of the first three epizootics and the rising number of fleas as breeding became established. The fifth and sixth epizootics differ from the others in that the mortality from plague is abnormally high in proportion to the flea prevalence. When, also, individual godowns are considered, the correspondence is not very close. Thus in godown 10, Experiments V and VI show a relatively excessive mortality: this is also seen in the sixth epizootic in godowns 11 and 12. In the fifth experiment the epizootic in godown 12 almost entirely failed though plenty of fleas were present.

2. *Septicaemia in the infecting rats.* The rats inoculated with plague which were introduced into each godown to start the infection were examined as soon as possible after death. Heart-blood smears were examined and the rats classified into three groups: (1) "low" when only an occasional bacillus was found in every few fields, (2) "moderate" when several bacilli were present in each field, and (3) "high" when every field showed large numbers of plague bacilli. Table II shows the results.

TABLE II.

Table showing comparative degree of septicaemia in the inoculated rats dying in the successive epizootics.

	1st epizootic	2nd epizootic	3rd epizootic	4th epizootic	5th epizootic	6th epizootic	7th epizootic	8th epizootic
Godown 10	Moderate	Low	High	Low	High	High	Low	Moderate
Godown 11	Low	Moderate	Moderate	Moderate	High	High	High	High
Godown 12	Low	Moderate	High	Low	High	High	Low	Moderate

Assigning the arbitrary values 1, 10 and 100 to the "low," "moderate" and "high" septicaemias respectively, we obtain the following rough numeric l comparison between the average degree of septicaemia and the mortality.

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TABLE III.

Number	Percent. mortality	Flea prevalence	Septicaemia
I	11	4	12
II	38	23	21
III	60	37	210
IV	42	16	12
V	62	21	300
VI	67	14	300
VII	53	22	102
VIII	36	16	120

The importance of the degree of septicaemia in the rats from which the fleas derive infection is obvious and its quantitative bearings on the possibility of transmission have been previously pointed out (vol. VII. p. 395, vol. VIII. pp. 286, 293). It has also been shown (vol. VI. p. 521) that rats killed during the later stages of the disease show an enormous variation in the degree of septicaemia; while the extreme variations in average flea prevalence in the different experiments are 4 and 37, the variation in the abundance of bacilli in the blood of the infecting rats may well be ten times as great. Be that however as it may, the fact remains that the highest average degree of septicaemia was found in experiments V and VI, where the mortality was high and the flea prevalence relatively low. Taking all the experiments in each godown separately, it appears that when the septicaemia was "low" 35 out of 105 (33 %) rats died in 7 epizootics, with "moderate" septicaemia 36 out of 105 (34 %) and with "high" septicaemia 95 out of 150 (63 %). It seems clear, therefore, that, though all the transmitting fleas do not in these experiments necessarily derive their infection from the inoculated rats put into the godowns, the observed differences in the degree of septicaemia afford some explanation of the varying severity of the different epizootics.

3. *Climatic conditions.* Continuous readings of temperature and humidity were kept in the godowns by means of thermographs and hygrographs. The temperature corresponds in some degree with the outside temperature in Bombay but the humidity throughout tends to be a good deal higher than the outside readings. This is due to the fact that the godowns were small and rather badly ventilated and, being lined with cement, they retained any moisture that got in during rainy weather.

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Table IV shows the maximum, minimum and mean weekly mean temperature and humidity inside the godowns during the experiment.

TABLE IV.

Experiment	Temperature	Humidity	Flea prevalence	Rat mortality %
I	76—80: 78° F.	83—96: 89% satn.	4	11
II	72—78: 75° ,,	70—85: 76 ,,	23	38
III	67—73: 71° ,,	72—88: 81 ,,	37	60
IV	70—75: 72° ,,	71—78: 75 ,,	16	42
V	81—84: 83° ,,	86—94: 88 ,,	21	62
VI	83—88: 85° ,,	91—99: 93 ,,	14	67
VII	80—82: 81° ,,	89—99: 92 ,,	22	53
VIII	75—79: 78° ,,	90—97: 94 ,,	16	36

The figures do not appear to show that the variations in temperature and humidity had a greater influence than other factors on the flea prevalence or the rat mortality[1].

4. *Relation to the natural epizootic.* During the period covered by the experiments, the prevalence of natural plague among the wild rats in Bombay was above the mean level from the first week in January to the third week in May. The first three experiments were made therefore during the earliest part of the natural epizootic, and they show an increasing flea prevalence associated with an increasing rat mortality. We have, however, already indicated that in the present experiments the flea prevalence was probably conditioned during the earlier experiments by the manner in which the godowns were stocked with fleas. The severe epizootics of Experiments V, VI and VII occurred during the decline of the natural epizootic and in the beginning of the off-plague season. We have previously recorded similarly severe experimental epidemics among guinea pigs during the off-plague season (vol. VII. p. 427), though the general correspondence between season and severity was more definite than in the present series (see also vol. VIII. p. 279).

5. *Susceptibility of the rats used.* We have shown (vol. VI. p. 505, VIII. p. 333, X. p. 458) that a considerable proportion (30—70 % according to the dose) of wild Bombay *rattus* possess a high degree of resistance to infection by hypodermic inoculation. In some of the present experiments (III, V, VI) as many as 60—67 % of the rats died, and it is questionable whether any mortality in excess of this

[1] Compare the series discussed in vol. VIII. p. 279 where, with less complete data, high temperatures appeared to have a considerable effect in limiting the epizootics.

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could be expected from flea transmission with these relatively immune animals. If that is so, once the conditions are sufficiently favourable to cause a mortality of this order, any further improvement in the facilities for the transmission of infection may lead to no corresponding increase of mortality. It should also be noted that, using 45 rats in each experiment, there is considerable room for accidental variations in mortality[1].

6. *Conclusions. As regards the mortality in the different epizootics it appears therefore that the flea prevalence and the degree of septicaemia developing in the infected rats were the chief influences at work.*

Disposal of the survivors of the epizootics.

A. *Examination for signs of resolving plague.* The survivors in the first three experiments were used.

TABLE V.

	Control godowns			Flea godowns		
Experiment	Examined	Resolving plague	Recovered plague	Examined	Resolving plague	Recovered plague
I	33	0	0	27	0	1
II	37	0	0	21	1	2
III	37	0	0	17	1	4
Totals	107	0	0	65	2	7

Two of the resolving plague lesions were shown to contain virulent plague bacilli. Of the seven examples of recovered plague, five were perisplenitis and two scars in the spleen (see vol. x. p. 335).

Of the survivors from the flea godowns therefore about 3 % showed resolving and 11 % recovered plague: of 107 survivors from the control flea-free experiments none showed any signs of having had an attack of plague.

B. *Resistance of the survivors of the epizootics.* The survivors of the fourth, sixth and seventh experiments were tested for their immunity by the hypodermic inoculation of an emulsion of plague rat's spleen.

[1] Thus in the fifth experiment in godown 10 all of the 15 rats died, in godown 11 12 out of 15, and in godown 12 only one out of 15. The 14 survivors from the last godown were afterwards inoculated with such a dose of plague as kills about 20 per cent. of ordinary Bombay *rattus*; all of them surviving, it is legitimate to suspect that they were not a fair sample.

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TABLE VI.

Experiment	Dose	Control godowns		Flea godowns	
		Inoculated	Died of plague	Inoculated	Died of plague
IV	1/5000 grm.	38	9	24	0
VI	1/5000 ,,	30	3	9	0
VII	1/2000 ,,	36	29	9	5

These figures show that the survivors among the rats which had been exposed to infection were much more immune than the control rats. In discussing the similar experiments which were previously made (vol. x. p. 332), it was pointed out that this could probably be accounted for by the elimination of most of the susceptible individuals during the experimental epizootics. In the present case this is not so clear, but there is no evidence of the rats having acquired immunity while in the godowns by the inoculation of small doses of *B. pestis* by fleas. That the immunity is only relative, is shown by the large number of deaths among the survivors of the seventh epizootic when a larger test dose of plague was given.

TABLE VII.

Distribution of the primary bubo in rats dying of plague in the godowns (see vol. VII. p. 382).

Cervical	147
Axillary	4
Cervical and axillary	1
Inguinal	2
Pelvic	2
No bubo	6
Eaten so that bubo could not be found	5
Total	167

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LV. OBSERVATIONS ON FLEA BREEDING IN POONA.

THE seasonal variations in the number of fleas per rat might depend either upon a variation in the number of their hosts, or a variation in the habits of the fleas themselves at different seasons of the year, or again upon the length of a flea's life at different times of the year, rather than upon the rate at which they breed. It was determined therefore to carry out these observations with a view to ascertaining what factors influence the rate of reproduction and the length of life of these insects, and to what extent any variations in their rate of reproduction or the length of their life might explain the seasonal variations in their numbers. These experiments with *X. cheopis* were commenced in July 1909 and were continued till February 1912, contemporary data as to the natural prevalence of fleas on *Mus rattus* in Poona being available throughout.

I. EGG LAYING.

(*a*) *Egg laying at different seasons of the year.*

Fleas were caught without the assistance of chloroform on freshly caught and killed rats. A number of adult females were selected and batches of five were put into test tubes and kept in a dark place. It was a difficult and slow process but it was found afterwards that a flea could easily be transferred from one test tube to another by means of a platinum needle, for after settling on the point it seldom jumped off in transference. As little time as possible was lost between the separation of these fleas from the rat and the commencement of the observations, as it was found that the largest number of eggs were laid during the first hour after separation from the rats. After being kept in a dark box for one hour the fleas were thrown away and the number of eggs deposited in the test tube were counted.

The result of the experiment will be seen in table I which compares in periods of one month the average number of eggs laid per flea with the average 8 a.m. humidity and mean temperature for

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that month from July 1909 to February 1912. These figures show that at any rate within certain limits of temperature the number of eggs laid by a flea is closely correlated to the atmospheric humidity.

TABLE I.

Table showing the average number of eggs laid per flea (X. cheopis) *in monthly periods. The average temperature and humidity in monthly periods is also shown.* (*Poona.*)

Month		Number of fleas	Number of eggs laid	Number of eggs per flea per hour	Average 8 o'clock humidity	Average monthly mean temperature
July	1909	145	231	1·6	84·4	75·3
Aug.	,,	180	423	2·4	81·9	75·9
Sept.	,,	225	467	2·1	82·2	75·5
Oct.	,,	230	457	2·0	71·8	77·0
Nov.	,,	255	339	1·3	55·6	74·9
Dec.	,,	245	331	1·4	62·3	70·8
Jan.	1910	240	317	1·3	52·1	69·7
Feb.	,,	198	335	1·7	39·3	72·0
March	,,	158	133	0·8	37·9	78·9
April	,,	127	79	0·6	30·3	84·5
May	,,	70	59	0·8	47·6	85·1
June	,,	111	185	1·7	73·2	79·8
July	,,	201	522	2·6	77·1	77·4
Aug.	,,	135	373	2·8	86·2	75·4
Sept.	,,	150	334	2·2	84·1	75·8
Oct.	,,	140	415	3·0	76·2	75·7
Nov.	,,	160	318	2·0	73·7	68·6
Dec.	,,	130	286	2·2	67·1	66·8
Jan.	1911	110	325	3·0	64·9	70·9
Feb.	,,	130	240	1·8	47·7	71·7
March	,,	125	187	1·5	52·5	76·8
April	,,	115	132	1·1	43·7	83·9
May	,,	120	86	0·7	52·0	87·3
June	,,	100	230	2·3	77·2	79·9
July	,,	135	411	3·0	75·6	78·4
Aug.	,,	130	314	2·4	85·5	74·9
Sept.	,,	120	335	2·8	79·9	76·4
Oct.	,,	95	244	2·6	75·4	81·0
Nov.	,,	100	204	2·0	66·9	76·4
Dec.	,,	90	218	2·4	62·9	71·6
Jan.	1912	70	144	2·1	57·7	71·3
Feb.	,,	50	101	2·0	64·0	74·3

In order to ascertain if possible the relative importance of temperature and humidity on the rate of egg laying the following experiments were carried out.

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(*b*) *Influence of varying humidity on egg laying at room temperature.*

This experiment was carried out to ascertain the number of eggs laid in one hour after removal from rats by fully grown female fleas in atmospheres of varying humidity. The fleas were divided into two batches and put into test tubes, a plug of cotton wool was inserted and this was closed with a rubber cap. One batch of fleas was kept under ordinary atmospheric conditions at room temperature and on the cotton wool plug of the other one or two drops of water was placed. The results of this experiment are shown in table II; it will be observed that in the presence of moisture, especially in the drier months of the year, a distinctly larger number of eggs were laid by the fleas.

TABLE II.

Table comparing the average number of eggs laid in one hour per flea in atmospheres having different degrees of humidity. (*Poona.*)

Month	Average 8 o'clock humidity	Average mean temperature	Normal			Moistened		
			Fleas	Eggs	Average	Fleas	Eggs	Average
January 1911	64·9	70·9	25	66	2·6	10	31	3·1
February ,,	47·7	71·7	50	53	1·1	15	32	2·1
March ,,	52·5	76·8	—	—	—	—	—	—
April ,,	43·7	83·9	20	26	1·3	20	47	2·3
	Total		95	145	1·5	45	110	2·4

(*c*) *Influence of varying temperature on egg laying.*

In this experiment, similar to the last, an attempt was made to ascertain the relative importance of temperature on the number of eggs laid in one hour after removal from their hosts by fully grown female fleas. In order to eliminate as far as possible the effect of relative humidity, which would vary at different temperatures, three test tubes were taken and between the rubber cap and cotton wool plug of each a piece of drying disc was placed consisting of asbestos impregnated with calcium chloride such as is used in the boxes in which photographic films are sold. These were kept for twenty-four hours in order to dry the air in the tube. The fleas were then put into these test tubes and kept in a dark place at various temperatures. One tube was kept at room temperature which varied between 24° and 28° C., another was placed in an incubator the temperature of which was from

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35° to 39° C. whilst a third was kept in a box containing some ice the temperature of which was from 13° to 20° C. The results show that this variation in the temperature has a marked effect on the number of eggs laid; it will be seen in table III that the room temperature was the most favourable for egg laying and that at both the higher and the lower temperatures fewer eggs were laid.

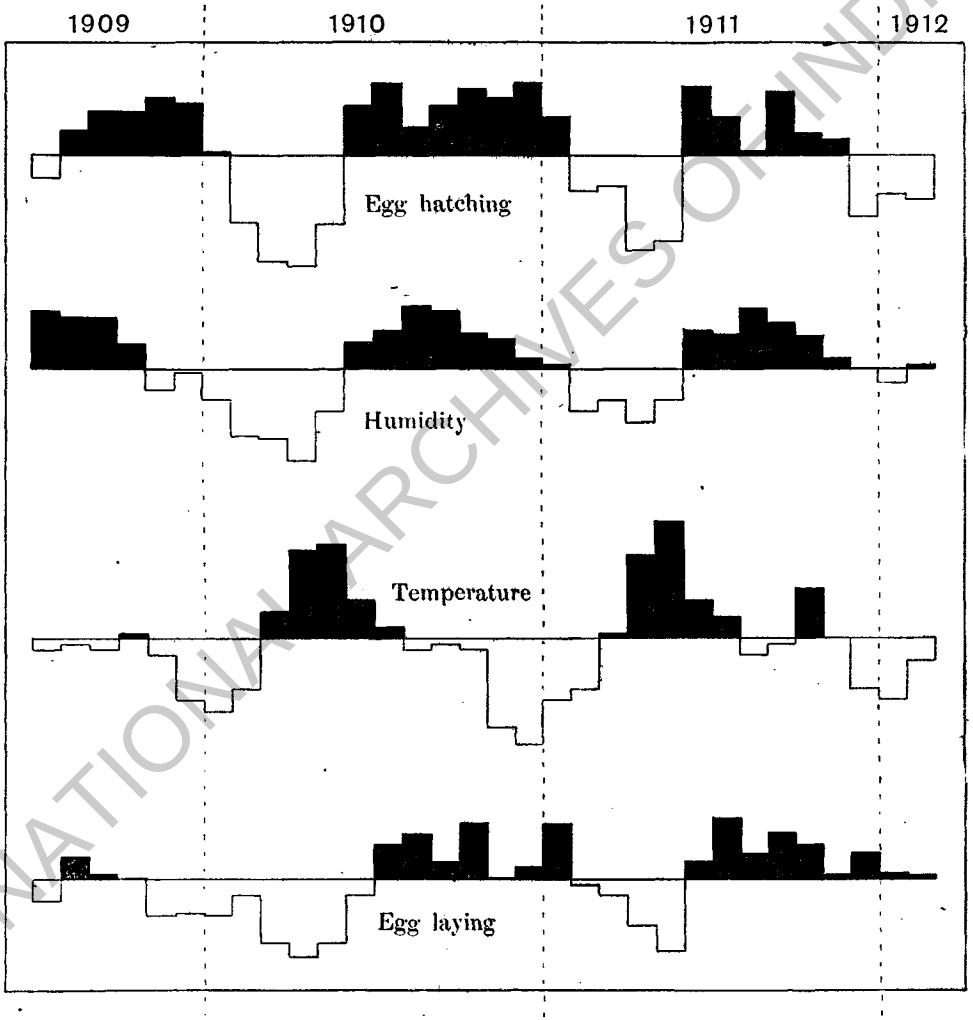

Fig. 1. The horizontal lines represent the mean values for 1910–11, and the ordinates the percentage variations for each month above and below these means.

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TABLE III.

Table showing the number of eggs laid at varying degrees of temperature when the effect of humidity has been as far as possible eliminated.

Date 1911–12	High Temperature: Mean temperature recorded	High Temperature: No. of fleas	High Temperature: No. of eggs laid	Room Temperature: Mean temperature recorded	Room Temperature: No. of fleas	Room Temperature: No. of eggs laid	Low Temperature: Mean temperature recorded	Low Temperature: No. of fleas	Low Temperature: No. of eggs laid	Remarks
Dec. 15	37	5	12	27	5	14	—	—	—	The atmosphere of the tubes was not previously rendered dry.
,, 16	35	5	9	—	—	—	20	5	5	
,, 18	38	5	3	25	5	11	20	5	11	
Jan. 1	39	5	1	—	—	—	18	5	2	Since January 1st atmosphere of the tubes was made dry prior to doing the experiment.
,, 4	38	5	—	24	5	10	14	5	—	
,, 5	39	5	—	24	5	14	—	—	—	
,, 6	37	5	—	24	5	12	13	5	—	
,, 12	—	—	—	24	5	2	14	5	—	
,, 17	39	5	—	29	5	6	15	5	—	
,, 19	39	5	—	28	5	10	15	5	—	
Average number of eggs per flea since Jan. 1, 1912	0·03			1·8			0·07			

II. Egg hatching.

(*a*) *Egg hatching at different seasons of the year.*

Test tubes in which fleas' eggs had been deposited were placed, some in a dark box and others in an ordinary well-lighted room, but not in direct sunlight, in order to note if the number of eggs hatched was in nature affected by daylight, and in addition to ascertain the number of eggs that hatch at different seasons of the year.

The result is shown in table IV. Approximately equal numbers of flea-eggs were kept in light and dark every month throughout the period of observation. Out of 5543 kept in light and 5571 kept in dark, the percentages hatched into larvae were 32·6 and 32·9 respectively. In the table the number of eggs hatched is compared with the average 8 a.m. humidity and it appears that, at any rate within certain limits of temperature, there is a distinct correlation between the number of eggs hatched at different seasons of the year and the percentage of atmospheric humidity.

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TABLE IV.

Table showing the percentage of flea-eggs hatched in monthly periods. The average monthly mean temperature and average 8 o'clock humidity are also shown for comparison.

Month		Number of eggs observed	Number of eggs hatched	Percentage of flea-eggs hatched	Average 8 o'clock humidity	Average monthly mean temperature
July	1909	381	83	20·9	84·4	75·3
Aug.	,,	454	145	31·9	81·9	75·9
Sept.	,,	467	170	36·3	82·2	75·5
Oct.	,,	443	161	36·5	71·8	77·0
Nov.	,,	325	128	39·5	55·6	74·9
Dec.	,,	327	128	38·7	62·3	70·8
Jan.	1910	346	93	26·4	52·1	69·7
Feb.	,,	384	38	10·6	39·3	72·0
March	,,	340	6	1·7	37·9	78·9
April	,,	161	nil	—	30·3	84·5
May	,,	179	20	10·2	47·6	85·1
June	,,	482	175	37·3	73·2	78·8
July	,,	621	268	43·2	77·1	77·4
Aug.	,,	731	238	32·6	86·8	75·4
Sept.	,,	577	220	38·1	84·1	75·8
Oct.	,,	609	256	42·0	76·2	75·7
Nov.	,,	467	185	39·6	73·7	68·6
Dec.	,,	624	268	42·9	67·1	66·8
Jan.	1911	497	177	35·6	64·9	70·9
Feb.	,,	390	70	17·9	47·7	71·7
March	,,	253	48	18·9	52·5	76·8
April	,,	178	6	3·4	43·7	83·9
May	,,	123	7	5·7	52·0	87·3
June	,,	271	115	42·4	77·2	79·9
July	,,	387	138	35·7	75·6	78·4
Aug.	,,	364	98	26·9	85·5	74·9
Sept.	,,	424	175	41·3	79·9	76·4
Oct.	,,	309	98	31·7	75·4	81·0
Nov.	,,	264	78	29·5	66·9	76·4
Dec.	,,	242	29	12·0	62·9	71·6
Jan.	1912	177	31	17·5	57·7	71·3
Feb.	,,	137	22	16·1	64·0	74·3

In table V the proportion of eggs hatching on successive days is shown, the period of incubation being longer during the cooler months.

In order to ascertain the relative importance of temperature and humidity on the rate of egg hatching, the following experiments were also carried out.

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TABLE V.

Table showing the percentage of eggs hatched to the total number hatched on successive days from the date on which they were laid.

Month		Average 8 o'clock humidity	Average mean temperature	Percentage of flea-eggs hatched	Percentage of flea-eggs hatched to the total hatched on successive days from date of keeping them												
					4	5	6	7	8	9	10	11	12	13	14	15	16
July	1909	84·4	75·3	21·8	—	32·5	36·1	26·5	3·6	1·2	—	—	—	—	—	—	—
Aug.	,,	81·9	75·9	31·9	—	49·7	44·1	4·8	0·7	0·7	—	—	—	—	—	—	—
Sept.	,,	82·2	75·5	36·4	8·8	25·9	51·8	10·0	2·9	0·6	—	—	—	—	—	—	—
Oct.	,,	71·8	77·0	36·3	2·5	54·7	39·8	2·5	0·6	—	—	—	—	—	—	—	—
Nov.	,,	55·6	74·9	39·1	—	6·2	28·9	35·2	22·7	5·5	1·6	—	—	—	—	—	—
Dec.	,,	62·3	70·8	39·1	—	—	30·5	60·2	6·2	2·5	0·8	—	—	—	—	—	—
Jan.	1910	52·1	69·7	26·9	—	—	—	40·9	30·1	19·4	9·7	—	—	—	—	—	—
Feb.	,,	39·3	72·0	9·9	—	—	10·5	68·4	7·9	5·3	5·3	—	3·6	—	—	—	—
March	,,	37·9	78·9	1·8	—	—	—	50·0	50·0	—	—	—	—	—	—	—	—
April	,,	30·3	84·5	—	—	—	—	—	—	—	—	—	—	—	—	—	—
May	,,	47·6	85·1	11·2	—	85·0	15·0	—	—	—	—	—	—	—	—	—	—
June	,,	73·2	70·8	36·3	11·4	45·1	34·9	5·7	2·3	0·6	—	—	—	—	—	—	—
July	,,	77·1	77·4	43·2	2·2	66·8	24·3	5·2	0·7	0·4	0·4	—	—	—	—	—	—
August	,,	86·8	75·4	32·6	—	35·7	42·0	17·2	4·2	0·8	—	—	—	—	—	—	—
Sept.	,,	84·1	75·8	38·1	—	46·4	35·0	12·3	0·5	0·9	0·5	—	—	—	—	—	—
Oct.	,,	76·2	75·7	42·0	—	36·7	53·1	9·4	—	0·4	—	—	—	—	—	—	—
Nov.	,,	73·7	68·6	39·6	0·5	0·5	25·0	10·0	8·7	14·6	14·6	11·9	—	1·1	0·5	1·5	1·1
Dec.	,,	67·1	66·8	42·9	—	—	—	3·4	39·1	41·4	13·4	1·5	1·1	—	—	—	—
Jan.	1911	64·9	70·9	35·6	—	—	19·2	45·8	19·8	5·6	9·0	0·6	—	—	—	—	—
Feb.	,,	47·7	71·7	17·9	—	—	15·7	35·7	21·4	5·7	7·1	7·1	—	7·1	—	—	—
March	,,	52·5	76·8	18·9	—	12·5	60·4	25·0	—	2·1	—	—	—	—	—	—	—
April	,,	43·7	83·9	3·4	—	66·6	33·3	—	—	—	—	—	—	—	—	—	—
May	,,	52·0	87·3	5·7	28·6	28·6	42·8	—	—	—	—	—	—	—	—	—	—
June	,,	77·2	79·9	42·4	11·4	71·3	13·0	1·7	2·6	—	—	—	—	—	—	—	—

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(b) Influence of a varying humidity on egg hatching.

Test tubes containing eggs were divided into three batches, and after being plugged with cotton wool were capped with india-rubber. In one batch the plug of cotton wool was moistened with a drop of water. In another one eighth of a Kodak film drying disc was placed, and the third was kept under the ordinary atmospheric conditions prevailing at the time. The results of these observations are seen in table VI

TABLE VI.

Table comparing the percentage of flea-eggs hatched into larvae at room temperature in test tubes having different degrees of humidity.

Month	Average 8 o'clock humidity	Average mean temperature	*A.* Normal			*B.* Moist			*C.* Dry		
			No. of eggs kept	No. of larvae found	Percentage	No. of eggs kept	No. of larvae found	Percentage	No. of eggs kept	No. of larvae found	Percentage
Jan. 1911	64·9	70·9	52	17	32·7	53	26	49·1	19	—	nil
Feb. ,,	47·7	71·7	57	15	26·3	29	12	41·4	41	—	nil
March ,,	52·5	76·8	43	10	23·3	41	16	39·0	—	—	—
April ,,	43·7	83·9	24	2	8·3	28	12	42·9	6	—	nil
May ,,	52·0	87·3	54	2	3·7	50	1	2·0	—	—	—
June ,,	77·2	79·9	156	69	44·2	39	7	17·9	23	2	8·7
July ,,	75·6	78·4	118	45	38·1	109	39	35·8	104	6	5·8
Aug. ,,	85·5	74·9	133	38	28·6	40	8	20·0	29	6	20·7
Sept. ,,	79·9	76·4	90	27	30·0	32	11	34·4	20	—	—
Totals	...	...	727	225	30·9	421	132	31·4	242	14	5·8
Average monthly percentage			—	—	26·1	—	—	31·4	—	—	3·9

A. Normal amount of humidity for the period (tube capped).

B. One drop of distilled water put on to the plug (tube capped).

C. One-eighth of a drying disc obtained from a roll of Kodak films placed inside the cotton plug (tube capped).

which shows that, compared with the tube kept under room conditions, the artificial raising of the humidity was distinctly favourable during the naturally dry months; but the difference was not so marked, indeed it was occasionally less favourable, in the months of the rainy season, especially in June and August. Artificial lowering of the humidity on the other hand was distinctly unfavourable in the dry season, when the atmospheric humidity was already very low. In the rainy season with this lowered humidity there was still a marked difference, except in the month of August when sufficient humidity

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was apparently not removed to interfere with the process of egg hatching. It was noticed that when the humidity was exceptionally high, moulds formed readily in the tubes, especially on the eggs, from which their mycelia could be seen radiating in every direction, and it is possible that these may have killed the eggs. It will be noticed

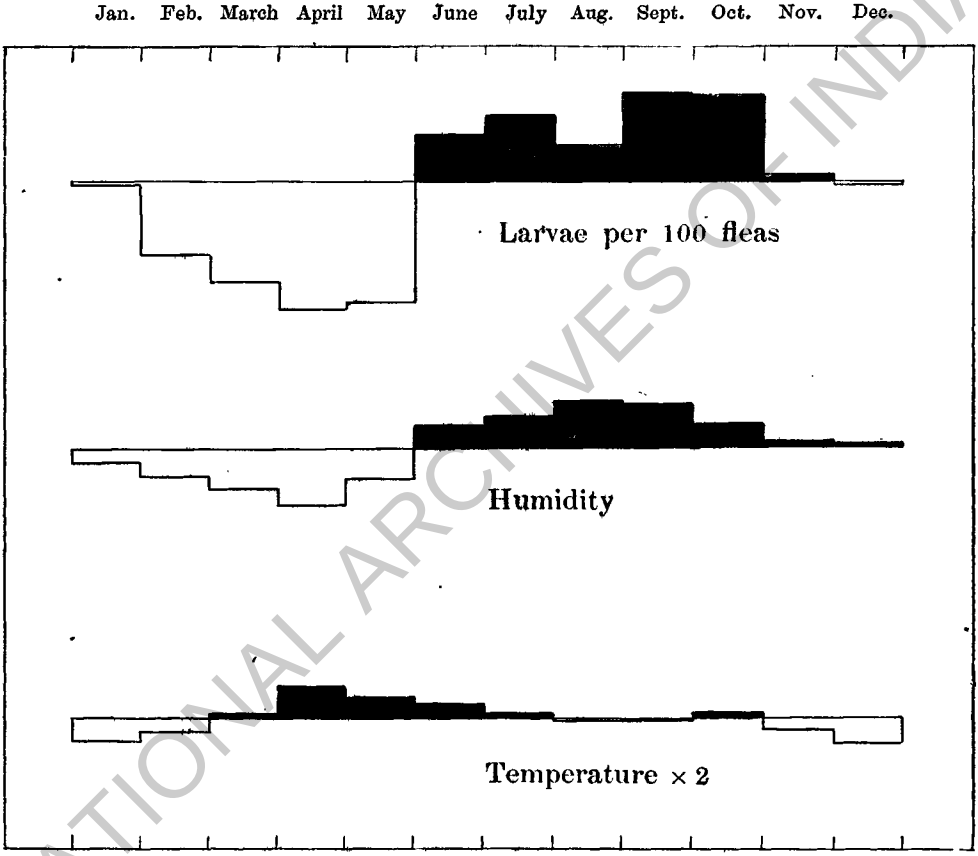

Fig. 2.

that in the month of August when the average humidity (85·5 %) was considerably higher than in any of the other months, fewer eggs were hatched under ordinary atmospheric conditions than there were during June, July and September. Moreover by further raising the humidity during this month the number of eggs hatched fell still further, that is, from 28·6 % to 20·0 %. The latter figure was even rather less than

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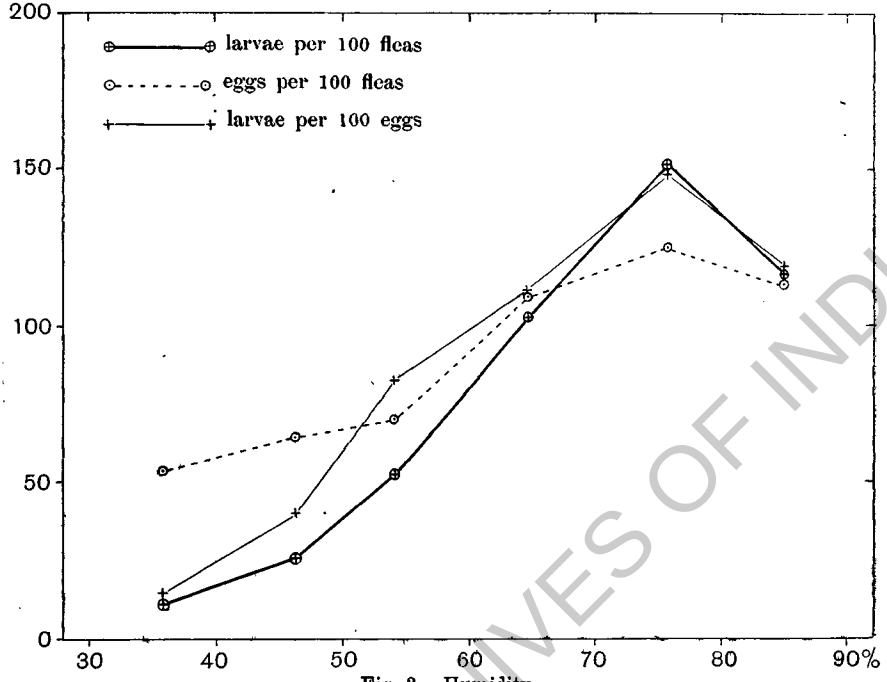

Fig. 3. Humidity

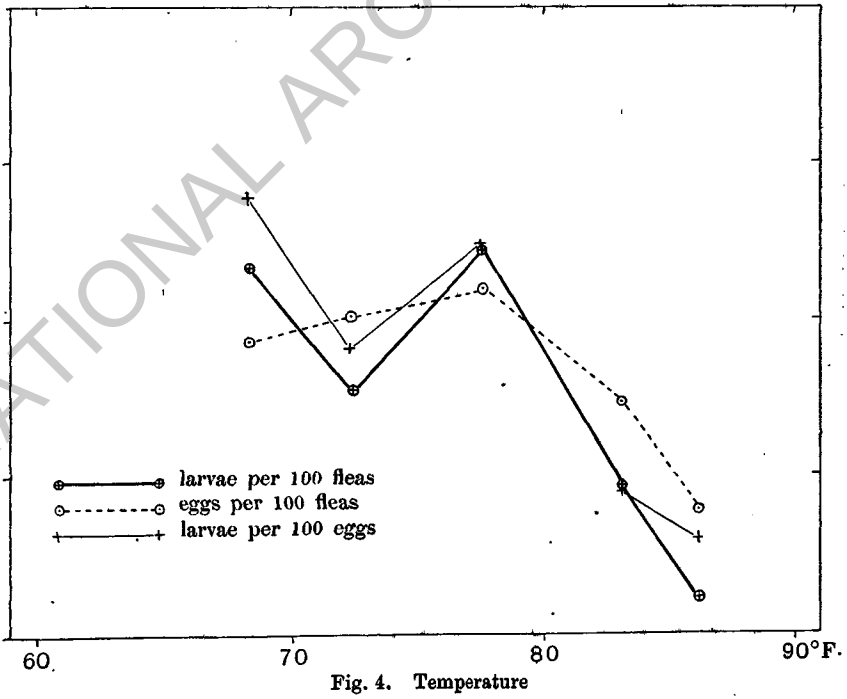

Fig. 4. Temperature

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that obtained in the "dry tube" which gave 20·7 % hatched. It would appear therefore that at the temperature then prevailing, *i.e.* about 74·9° F., an average atmospheric humidity above 85 is already detrimental to the hatching of fleas' eggs. When this humidity is further raised it becomes distinctly more unfavourable. It is probable that, in this month, even under ordinary atmospheric conditions the formation of moulds, as we have already explained, may have interfered considerably with the hatching of eggs, but whether this occurs also in nature we cannot tell.

(*c*) *Influence of varying temperature on egg hatching.*

A variation in the temperature naturally affects the percentage of atmospheric humidity; it was therefore difficult to keep the latter at a constant figure over a period such as is required for the hatching of eggs, and the further development of these through the various stages in the life history of the flea. An attempt was made to eliminate the humidity factor by drying the air in each test tube by means of drying discs, but in this case none of the 61 eggs kept at a higher temperature, nor 51 kept at the room temperature, nor 36 kept at a low temperature hatched into larvae.

(*d*) The number of eggs laid per 100 fleas (in an hour) being recorded for each month during our observations as well as the number of these that hatched into larvae, the number of larvae obtained from the eggs laid by every 100 fleas kept for one hour could be ascertained. This recorded for each month is shown in table VII, together with the egg laying and hatching figures separately. In order that the three series may be readily compared, the data have been also calculated relative to the average figures for the complete double cycle from January 1910 to December 1911 taken as 100. These summary figures show very plainly that the seasonal changes in egg laying and in egg hatching run together with the result that more than twenty times as many larvae were obtained in September and October, when it was warm and wet, as in April and May, when it was hot and dry.

In Poona the mean temperature varies comparatively little at different seasons of the year, and much less than the humidity. The hottest months are also the driest though in the cooler season the humidity is not at its maximum. It is a matter of some interest to disentangle as far as possible the influence of humidity from that of

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TABLE VII.

Table showing the number of larvae obtained from the eggs laid in one hour by a hundred fleas in monthly periods.

Month		Eggs per 100 fleas		Percentage of eggs hatched		Larvae per 100 fleas	
		Absolute	Relative*	Absolute	Relative*	Absolute	Relative*
July	1909	159	80	20·9	80	33	55
August	,,	235	**119**	31·9	**122**	75	**125**
September	,,	208	**105**	36·3	**139**	75	**125**
October	,,	199	**100**	36·5	**139**	73	**122**
November	,,	133	67	39·5	**151**	53	88
December	,,	135	68	38·7	**147**	52	87
January	1910	132	67	26·4	**101**	35	58
February	,,	169	85	10·6	40	18	30
March	,,	84	42	1·7	6	1	2
April	,,	62	31	0·0	0	0	0
May	,,	84	42	10·2	39	9	15
June	,,	167	84	37·3	**143**	62	**103**
July	,,	260	**131**	43·2	**165**	112	**120**
August	,,	276	**140**	32·6	**125**	90	**150**
September	,,	223	**113**	38·1	**145**	84	**140**
October	,,	296	**150**	42·0	**161**	124	**207**
November	,,	199	**100**	39·6	**151**	79	**132**
December	,,	220	**111**	42·9	**164**	94	**157**
January	1911	295	**149**	35·6	**136**	105	**175**
February	,,	185	93	17·9	68	33	55
March	,,	150	76	18·9	72	28	47
April	,,	115	58	3·4	13	4	7
May	,,	72	36	5·7	22	4	7
June	,,	230	**116**	42·4	**162**	98	**163**
July	,,	304	**153**	35·7	**136**	109	**182**
August	,,	242	**122**	26·9	**103**	65	**108**
September	,,	279	**141**	41·3	**158**	115	**192**
October	,,	257	**130**	31·7	**121**	81	**135**
November	,,	204	**103**	29·5	**113**	60	**100**
December	,,	242	**122**	12·0	46	29	48
January	1912	206	**104**	17·5	67	36	60
February	,,	202	**102**	16·1	61	32	53
Average Jan. 1910 to Dec. 1911		198	100	26·2	100	60	100

* Relative to the mean values from January 1910 to December 1911.

temperature. To some extent this may be done with the figures given in table VII. If these are grouped according to humidities (table VIII), there is a regular increase in larva production up to 80% saturation which shows no particular relation to the average temperature of each group of months. The fall in the most humid group, with a medium temperature, is perhaps associated with the excessive growth of moulds

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under these conditions which has been already mentioned. On the other hand, the same figures grouped according to the dry bulb thermometer (table IX) show far less regularity. On the whole therefore it appears

TABLE VIII.

Mean relative figures of table VII grouped by humidities.

	Group	No. of months	Mean humidity	Mean temperature	Eggs per 100 fleas	Percent. hatched	Larvae per 100 fleas
I	30—40 %	3	35·8	78·5	53	15	11
II	40—50 %	3	46·3	80·2	64	40	26
III	50—60 %	5	54·0	76·0	70	83	52
IV	60—70 %	6	64·7	71·6	109	111	103
V	70—80 %	9	75·6	77·1	123	148	151
VI	80—90 %	6	85·0	75·5	113	119	117

TABLE IX.

Mean relative figures of table VII grouped by temperatures.

	Group	No. of months	Mean humidity	Mean temperature	Eggs per 100 fleas	Percent. hatched	Larvae per 100 fleas
I	65—70°	3	64·3	68·4	93	139	116
II	70—75°	9	60·0	72·4	101	91	78
III	75—80°	15	74·2	77·6	110	124	122
IV	80—85°	3	49·8	83·1	73	45	47
V	85—90°	2	49·8	86·2	39	30	11

TABLE X.

Month	Humidity	Temperature	Eggs per 100 fleas	Percent. hatched	Larvae per 100 fleas
March 1910	37·9	78·9	42	6	2
„ 1911	52·5	76·8	76	72	47
Nov. „	66·9	76·4	103	113	100
Oct. 1909	71·8	77·0	100	139	122
June 1910	73·2	79·8	84	143	103
July 1911	75·6	78·4	153	136	182
Oct. 1910	76·2	75·7	150	161	207
July „	77·1	77·4	131	165	120
June 1911	77·2	79·9	116	162	163
Sept. „	79·9	76·4	141	158	192
Aug. 1909	81·0	75·9	119	122	125
Sept. 1910	84·1	75·8	113	145	140
July 1909	84·4	75·3	80	80	55
Aug. 1910	86·8	75·4	140	125	150
Sept. 1909	88·2	75·5	105	139	125

likely that, with the range of temperature obtaining in Poona, the seasonal variation in larva production in these experiments was conditioned more by the favouring influence of a high humidity than by the destructive effect of an excessive temperature. Much the same is indicated by the figures for the individual months in any one group.

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Table X gives the figures for the fifteen months in which the temperature fell between 75·0° and 79·9° while the humidity ranged from 38 % to 88 %.

Table VII also appears to indicate that there is a greater seasonal variation in the percentage of eggs hatching into larvae than in the number of eggs laid. Since both these stages have a seasonal variation in the same sense, the number of larvae obtained per flea, which is the product of them both, naturally shows the widest range[1]. In the artificially humidified experiments there is the same difference between normal and moist conditions in egg laying (table II) as in egg hatching (table VI).

III. The development of the larvae into pupae and into fully formed fleas.

(*a*) *Seasonal variation.*

These observations were commenced in August 1910. The larvae when hatched out of eggs were kept in test tubes and fed on wheat bran. Since the middle of July 1911 an attempt was made to sterilize

TABLE XI.

Table showing the number and percentage of fleas formed from the larvae of Xenopsylla cheopis *allowed to feed on wheat bran at room temperature and at normal atmospheric humidity.*

Month	Average mean temperature	Average 8 o'clock humidity	No. of larvae kept	No. of fleas formed	Percentage of fleas formed
Aug. 1910	75·4	86·8	133	16	12·0
Sept. ,,	75·8	84·1	167	8	4·8
Oct. ,,	75·7	76·2	107	0	nil
Nov. ,,	68·6	73·7	45	0	nil
Dec. ,,	66·8	67·1	79	0	nil
Jan. 1911	70·9	64·9	152	0	nil
Feb. ,,	71·7	47·7	72	0	nil
March ,,	76·8	52·5	44	0	nil
April ,,	83·9	43·7	7	0	nil
May ,,	87·3	52·0		No larvae kept	
June ,,	79·9	77·2	40	9	22·5
July ,,	78·4	75·6	94	19	20·2
Aug. ,,	74·9	85·5	56	7	12·5
Sept. ,,	76·4	79·9	88	5	5·7
Oct. ,,	81·0	75·4	59	0	nil
Nov. ,,	76·4	66·9	22	0	nil
Dec. ,,	71·6	62·9	32	0	nil

[1] The method is not altogether valid in the present instance, but we may note that the variabilities (coefficient of variation) of the figures in table VII are 34, 50 and 63 for egg laying, egg hatching and larvae respectively.

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the bran by heating. The movements of the larvae and the formation of cocoons were noted from time to time, and the further development of the young fleas was watched. The results are shown in table XI. It will be seen that the greatest percentage of fleas were developed from larvae during June and July, fewer were found in August and very few in September, whilst none were found during the remaining months of the year. No larvae were kept for observation during May as none were available[1].

(*b*) *The influence of varying humidity on the development of larvae.*

The larvae were kept in two batches in test tubes plugged with cotton wool and capped with a rubber cap. Small pieces of blotting paper 2 mm. square were put into each: in the one case they were dry, and in the other they were moistened with water. The larvae were then kept under observation in a dark box as already described. The result will be seen in table XII. It will be noticed that in the normal atmosphere cocoons were visible only during the months of June to October inclusive, and fleas were found only from June to September inclusive. It will be seen that the moist atmosphere however was considerably more favourable to the life of the larvae and their development into pupae and fleas than a dry atmosphere. It is of interest to note that during the humid months of August and September—which under normal conditions are favourable to the development of pupae and larvae—when the humidity was still further raised, it appears to be detrimental to their development, for comparatively few fleas were found. This is probably due to the growth of the moulds, for the bran was on several occasions seen to be mouldy during these months. These figures must be taken with some caution for it must be remembered that, as we have already mentioned, our results in this experiment were at certain seasons unfortunately based on very small figures.

[1] It is to be regretted that during the drier months of the year these results are based on very small figures. This was due to the fact that the number of fleas during these months was very limited, and moreover as we have seen very few eggs also were laid, and scarcely any of these were hatched in the course of our experiments.

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TABLE XII.

Table comparing the development of larvae (Xenopsylla cheopis) *into cocoons and fleas when at room temperature and at different degrees of humidity.*

		A. Normal atmospheric condition						B. Moist					
				Cocoons visible		Fleas formed				Cocoons visible		Fleas formed	
Month, 1911	Average 8 o'clock humidity	Number of larvae kept	Mean day till which larvae were active	Number formed	Percentage of larvae kept	Number formed	Percentage of larvae kept	Number of larvae kept	Mean day till which larvae were active	Number formed	Percentage of larvae kept	Number formed	Percentage of larvae kept
January	64·9	94	18·2	—	—	—	—	95	24·2	4	4·2	4	4·2
February	47·7	78	7·5	—	—	—	—	76	20·2	6	7·9	9	11·8
March	52·5	44	7·0	—	—	—	—	42	24·5	3	7·1	6	14·3
April	43·7	7	10·0	—	—	—	—	5	10·5	—	—	—	—
May	52·0	—	—	—	—	—	—	—	—	—	—	—	—
June	77·2	40	28·0	1	2·5	9	22·5	38†	25·7	2	5·3	12	31·6
July*	75·6	94	31·6	13	13·8	19	20·2	91‡	24·4	10	11·0	12	13·2
August	85·5	56	32·7	7	12·5	7	12·5	56§	28·5	—	—	1	1·8
September	79·9	88	40·7	5	5·7	5	5·7	54	48·0	2	3·7	1	1·9
October	75·4	59	10·0	1	1·7	—	—	59	31·5	5	8·5	1	1·7
November	66·9	22	10·7	—	—	—	—	21	24·7	1	4·8	—	—
December	62·9	32	11·7	—	—	—	—	31	25·5	—	—	—	—
	*	614	19·0	27	4·4	40	6·5	568	26·2	33	5·8	46	8·1

A. A capped glass test tube representing normal amount of humidity for the period. A 2 millimetre square piece of ordinary blotting paper put inside bran as a control.

B. A capped test tube having humidity artificially raised above the normal by putting a 2 mm. square piece of moistened blotting paper inside the bran.

* Since the middle of this month, the bran in which the larvae were fed was sterilized by dry heat.

† The bran in which 23 of these larvae were fed became mouldy.

‡ ,, ,, 75 ,, ,, ,, ,,

§ ,, ,, 32 ,, ,, ,, ,,

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TABLE XIII.

Table showing the average life in days of a rat flea when kept isolated in glass test tubes without feeding.

Half-month ending		Number of fleas observed	Number of days lived	Average life in days	Average 8 o'clock humidity
15 July	1910	65	194·0	2·98	77·4
31 ,,	,,	55	158·0	2·88	76·8
15 August	,,	90	262·5	2·92	87·5
31 ,,	,,	65	197·5	3·04	86·0
15 September	,,	50	173·5	3·46	82·1
30 ,,	,,	25	61·0	2·44	86·0
15 October	,,	60	150·0	2·50	79·9
31 ,,	,,	70	224·5	3·20	72·5
15 November	,,	45	127·0	2·82	77·4
30 ,,	,,	45	114·5	2·54	69·9
15 December	,,	60	162·5	2·70	64·2
31 ,,	,,	60	188·5	3·14	70·0
15 January	1911	45	147·0	3·26	67·1
31 ,,	,,	40	111·0	2·78	62·6
15 February	,,	50	102·5	2·04	45·5
28 ,,	,,	70	98·0	1·40	49·9
15 March	,,	45	72·5	1·62	52·5
31 ,,	,,	65	70·0	1·08	46·5
15 April	,,	65	59·5	0·92	44·8
30 ,,	,,	55	40·5	0·74	42·5
15 May	,,	45	44·0	0·98	45·9
31 ,,	,,	45	61·5	1·36	52·1
15 June	,,	45	135·5	3·02	80·2
30 ,,	,,	40	165·0	4·12	74·3
15 July	,,	55	172·5	3·14	77·4
31 ,,	,,	50	134·0	2·68	73·8
15 August	,,	55	256·0	4·66	84·7
31 ,,	,,	45	196·0	4·36	86·4
15 September	,,	35	120·5	3·44	83·3
30 ,,	,,	35	126·0	3·60	76·5
15 October	,,	40	116·0	2·92	79·0
31 ,,	,,	45	101·0	2·24	71·9
15 November	,,	45	75·5	1·68	58·6
30 ,,	,,	40	160·5	4·02	75·3
15 December	,,	35	132·0	3·78	68·0
31 ,,	,,	60	182·0	3·04	57·9
15 January	1912	40	82·5	2·06	59·4
31 ,,	,,	35	70·5	2·02	56·0
15 February	,,	25	60·5	2·42	74·5
28 ,,	,,	25	50·5	2·02	64·0
15 March	,,	20	17·0	0·85	56·6

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IV. The length of life of a flea[1].

(*a*) *Seasonal variations.*

If fleas conveyed in luggage or merchandise (such as grain) may be the means of transmitting plague from one place to another over considerable distances, the length of the life of a flea whilst so confined (*i.e.* without feeding), if found to vary much at different seasons, might be a factor of considerable importance, favourable or otherwise, to the spread of plague from village to village or from one district to another. On the facility with which plague infection can be so transported, the severity of an epidemic in a district would also largely depend.

With a view to determining what seasonal variations, if any, there are in the length of life of a flea the following observations were undertaken. Fully grown fleas were selected and kept in capped glass test tubes together with small pieces of cloth. The number dying on successive days was noted. The average length of life of a flea was then calculated in periods of half a month.

The results are shown in table XIII compared with the half-monthly average 8 a.m. humidity[2]. It will be seen that there is a marked correlation between the length of their life and the humidity. The longest life is during August when the humidity is over 80 % and their shortest life in April and the first half of May, when the humidity is about 45 % or less. It is of interest to note that in August they were, under the conditions observed in the experiment, found to live

TABLE XIV.

Table showing the average number of days which one rat flea lived when kept isolated in glass test tubes without feeding.

Half-month ending	Number of fleas kept	Total life	Average	Average 8 o'clock humidity (half-monthly)	Average half-monthly mean temperature
March 15, 1911	15	27·5	1·8	58·5	74·6
,, 31 ,,	10	2·0	0·2	46·5	79·0
April 15 ,,	25	22·5	0·9	44·8	83·1
,, 30 ,,	20	12·0	0·6	42·5	84·7
May 15 ,,	25	10·0	0·4	45·9	87·6
,, 31 ,,	50	37·5	0·7	58·1	87·0
June 15 ,,	30	75·5	2·5	80·2	80·7
,, 30 ,,	25	50·5	2·0	74·3	79·1

[1] For observations on *Ceratophyllus fasciatus* see W. Nicoll, *Brit. Med. Journ.* 1912, vol. II. p. 926.

[2] The monthly temperatures and humidities are given in table I above.

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about five times longer than they do in April, in other words, plague-infected fleas could under similar conditions be conveyed five times farther in August than they could be in April. Table XIV similarly gives the length of life of unselected fleas. It will be seen that a similar variation takes place if full-sized fleas are not selected.

(*b*) *Influence of varying humidity.*

In a manner similar to the previous experiments already described, fleas were put into test tubes and divided into three batches. In one the humidity was artificially raised by means of a drop of water, in another it was lowered by means of a piece of a photographic drying disc, the third batch being kept capped under the atmospheric conditions of the room. The result is found in table XV; it will be seen that, in almost every month, the effect of the addition of moisture prolonged the life of the flea, the contrast was most marked in the months of the hot dry weather. The abstraction of moisture, on the other hand, almost

TABLE XV.

Table comparing the average life in days of X. cheopis *kept in the dark at room temperature in glass test tubes having different degrees of humidity.*

Month		Average 8 o'clock humidity	Average mean temperature	*A* Normal			*B* Moist			*C* Dry		
				Fleas kept	Total life in days	Average life of one flea in days	Fleas kept	Total life in days	Average life of one flea in days	Fleas kept	Total life in days	Average life of one flea in days
Jan.	1911	64·9	70·9	15	55·0	3·7	15	61·5	4·1	10	13·5	1·3
Feb.	,,	47·7	71·7	10	17·5	1·7	10	44·0	4·4	5	11·0	2·2
March	,,	52·5	76·8	10	6·0	0·6	15	55·0	3·7	15	6·0	0·4
April	,,	43·7	83·9	10	4·5	0·4	10	19·5	1·9	—	—	—
May	,,	52·0	87·3	No observations made								
June	,,	77·3	79·9									
July	,,	75·6	78·4	15	43·5	2·9	10	29·5	2·9	10	10·0	1·0
Aug.	,,	85·5	74·9	45	192·5	4·3	35	254·5	7·3	30	59·0	1·9
Sept.	,,	79·9	76·4	20	70·0	3·5	10	58·0	5·8	10	13·0	1·3
Oct.	,,	75·4	81·0	15	45·0	3·0	15	57·5	3·8	10	7·0	0·7
Totals		—	—	140	434·0	3·1	120	579·5	4·8	90	119·5	1·3

A. Without any moisture or drying disc (tube capped).

B. One drop of distilled water added on to the cotton plug (tube capped).

C. One-eighth of a drying disc obtained from a roll of Kodak films, placed inside the cotton plug (tube capped).

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always shortened the length of the life of a flea. The only exception occurred in the month of February but this may be accounted for by the fact that the figures were too small to strike an average, as in this month observations were made on only five fleas kept in a dry atmosphere.

(*c*) *Influence of varying temperature.*

It was somewhat difficult to keep the humidity constant under varying conditions of temperature for a period extending over several days, so in a manner similar to the experiment (p. 302) already described, an attempt was made to eliminate the effect of moisture by means of a small piece of photographic drying disc kept in the tubes. The tubes containing fleas were then kept under varying conditions of temperature, and the length of life of the flea was observed in the usual manner.

TABLE XVI.

Table showing the average life of one full grown cheopis *kept without feeding at different temperatures, the atmosphere of the tubes having been rendered dry by a piece of drying disc placed inside them prior to capping.*

Date	*A* Incubator 98°—104° F. = 101° F.		*B* Room 55°—85° F. = 70° F.		*C* Ice-box 55°—65° F. = 60° F.	
	Fleas kept	Average life of one in days	Fleas kept	Average life of one in days	Fleas kept	Average life of one in days
December 15, 1911	5	0·0	5	1·5	5	2·2
,, 16 ,,	5	0·5	5	0·5	5	1·1
,, 18 ,,	5	0·0	5	1·0	5	1·4
,, 20 ,,	5	0·0	5	0·2	5	0·2
,, 21 ,,	5	0·0	5	1·4	5	1·2
,, 22 ,,	5	0·0	5	2·4	5	2·2
,, 26 ,,	5	0·0	5	1·0	5	1·6
,, 29 ,,	5	0·3	5	1·5	5	2·1
,, 30 ,,	5	0·5	5	1·0	5	1·6
January 4, 1912	5	0·0	5	1·3	5	2·2
,, 6 ,,	5	0·5	5	1·1	5	2·2
,, 23 ,,	5	0·0	0	0	5	1·2
February 2 ,,	5	0·0	0	0	5	2·4
Total fleas kept and the mean average life of one flea	65	0·14	55	1·17	65	1·66

The result is seen in table XVI. It will be seen that the average length of life of the flea was considerably longer in the low temperature

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(55°—65° F.) than in the room temperature (55°—85° F.); in this again it was considerably longer than in the warm temperature 98°—104° F.

An analysis of the monthly figures for 20 months given in table XIII with reference to humidity and temperature is shown in tables XVII and XVIII and graphically in figures 5 and 6. The relation to humidity appears to be more regular and definite than to temperature.

TABLE XVII.

Average monthly figures of table XIII grouped by humidities.

Group		No. of months	Mean humidity	Mean temperature	Average duration of life
I	30—40 %	0	—	—	—
II	40—50 %	2	45·7 %	77·8°	1·27
III	50—60 %	3	54·1	78·5	1·52
IV	60—70 %	5	65·1	71·8	2·88
V	70—80 %	7	76·4	76·8	3·01
VI	80—90 %	3	85·5	75·4	3·48

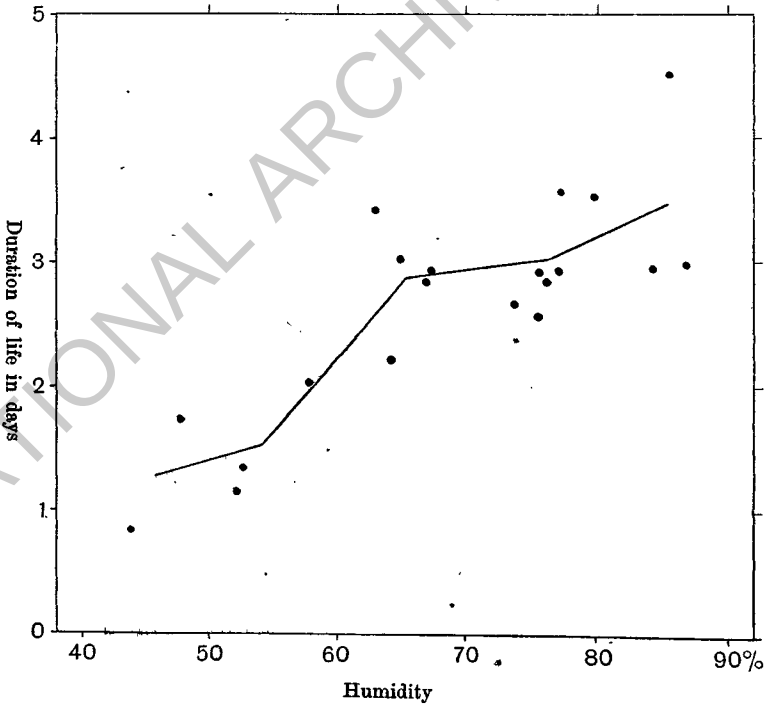

Fig. 5.

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TABLE XVIII.

Average monthly figures of table XIII grouped by temperatures.

Group		No. of months	Mean humidity	Mean temperature	Average duration of life
I	65—70°	2	70·4 %	67·7°	2·80
II	70—75°	6	63·8	72·3	2·82
III	75—80°	9	75·1	76·9	2·88
IV	80—85°	2	60·0	82·5	1·70
V	85—90°	1	52·0	87·3	1·35

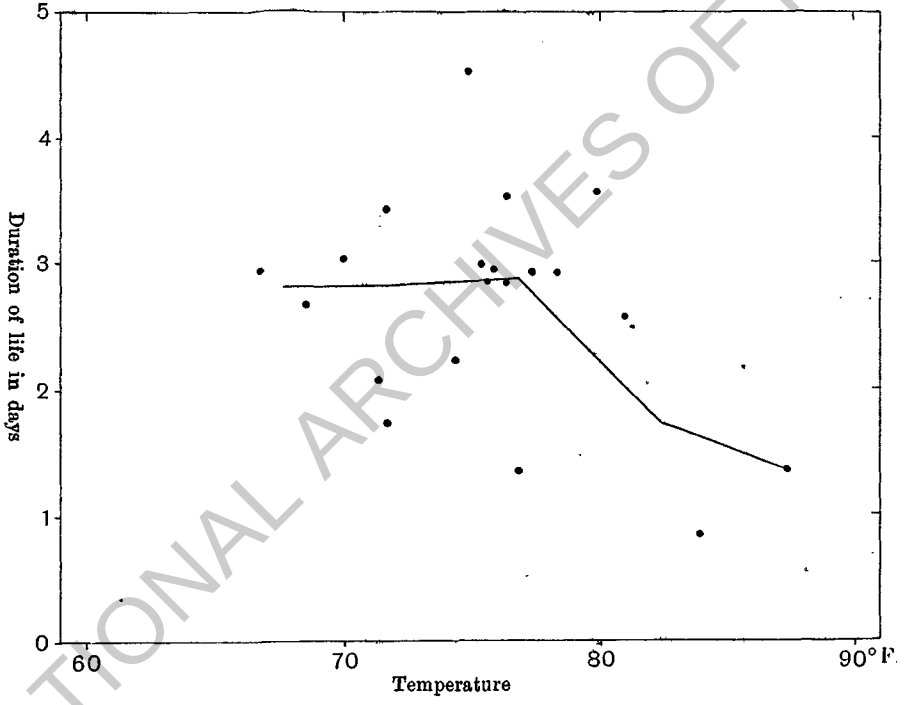

Fig. 6.

(*d*) *The length of life of a newly-hatched flea.*

Newly-hatched and fully-developed fleas were kept in test tubes without feeding and the length of their lives noted. The result will be seen in table XIX to be that, under the conditions of the experiment, the length of life of a newly-hatched flea that has never fed is considerably longer than that of an adult.

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TABLE XIX.

Table comparing the average life of a fully-grown flea with that of a newly-hatched flea under similar conditions.

Half-month ending	Fully-grown flea			Newly-hatched flea		
	Fleas kept	Total life in days	Average	Fleas kept	Total life in days	Average
28 February, 1911	70	98·0	1·4	1	5·0	5·0
15 March ,,	45	72·5	1·6	3	12·5	4·2
31 ,, ,,	65	70·0	1·1	1	7·0	7·0
15 May ,,	45	44·0	1·0	1	2·0	2·0
15 July ,,	55	172·5	3·1	9	81·5	9·1
31 ,, ,,	50	134·0	2·7	13	97·0	7·5
15 August ,,	60	282·0	4·7	9	75·0	8·3
31 ,, ,,	45	196·0	4·4	12	116·0	9·7
15 September ,,	35	120·5	3·4	13	57·5	4·4
30 ,, ,,	35	126·0	3·6	1	3·0	3·0
15 October ,,	40	116·5	2·9	1	4·0	4·0
Total	545	1432	2·6	64	460·5	7·2

V. Relation to natural prevalence of fleas.

Observations on the natural prevalence of fleas on wild *M. rattus* in Poona were made by the usual method continuously from July 1908 to February 1912. Some of the figures have been already published

TABLE XX.

Month	Fleas per 100 rats		Relative figures for						
	Absolute	Relative*	Egg laying	Egg hatching	Larvae	Length of life	Humidity	Temperature	Average relative weekly plague mortality†
Jan.	433	90	**107**	**101**	98	**105**	58·2 %	70·3°	**156**
Feb.	437	91	93	57	46	82	50·3	72·7	**103**
March	397	83	59	39	25	49	45·2	77·8	35
April	290	61	44	6	3	34	37·0	84·2	12
May	213	45	39	30	10	48	49·8	81·2	7
June	240	50	**100**	**152**	**133**	**148**	75·2	79·8	8
July	520	**109**	**122**	**127**	**141**	**121**	79·0	77·0	45
August	825	**173**	**127**	**116**	**128**	**155**	84·4	75·4	**140**
Sept.	880	**184**	**119**	**147**	**153**	**134**	84·1	75·9	**200**
October	765	**160**	**127**	**140**	**155**	**112**	74·5	77·9	**203**
Nov.	540	**113**	91	**138**	**106**	**114**	65·4	73·3	**151**
Dec.	480	**100**	**100**	**119**	98	**131**	64·1	69·7	**139**

* To the average monthly flea prevalence (479) for the 24 months, January 1909 to December 1910.

† See Vol. x. p. 495.

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(Vol. x. p. 525), and the whole series will be described in detail in a subsequent report. Neglecting for the moment the interannual variations, we give in table XX the average monthly figures for the whole period, comparing them with the data obtained by artificial breeding.

There is thus a good correspondence between the results of artificial breeding and the prevalence of fleas in nature. The marked increase in egg laying and hatching occurs in June with the onset of wet weather and the prevalence of imagines in nature follows in July.

VI. Summary.

1. *The laying of eggs by wild fleas, the development of the eggs into larvae and the development of larvae into pupae and imagines all show a marked seasonal variation, being most active when the weather is wet and the temperature moderate and least active under dry and hot conditions.*

2. *Within the range of temperatures obtaining in Poona, atmospheric humidity seems to be a more important factor than temperature in conditioning this seasonal variation.*

3. *Artificial humidification of the atmosphere is favourable to all stages of the metamorphosis especially in the dry season.*

4. *The seasonal variations in the results of artificial breeding correspond in time to the seasonal variations in the prevalence of fleas on rats in nature.*

5. *The life of an adult flea is longer in a cool and moist atmosphere than under hot and dry conditions.*

Owing to the failure to breed out imagines except in the most favourable months, it is not possible to give any numerical expression to the seasonal variation in the abundance of the second generation of fleas. Since the whole life history of the flea in Poona—where the weather is never cold—is conditioned in the same sense by atmospheric changes, it is clear that the numbers of the second generation are the product of the activity of the various phases. In July for example with a humidity of 79 % and a temperature of 77°, 100 fleas lay 241 eggs of which 33 % hatch; of the resulting 80 larvae, 20 % develop successfully and the 16 fleas obtained, living on the average three days, would give 48 flea-days. In April, on the other hand, the humidity being 37 % with a temperature of 84°, 100 fleas yielded only 88 eggs and these only 1·5 larvae: assuming that 1 % of these developed, we get 0·015 fleas

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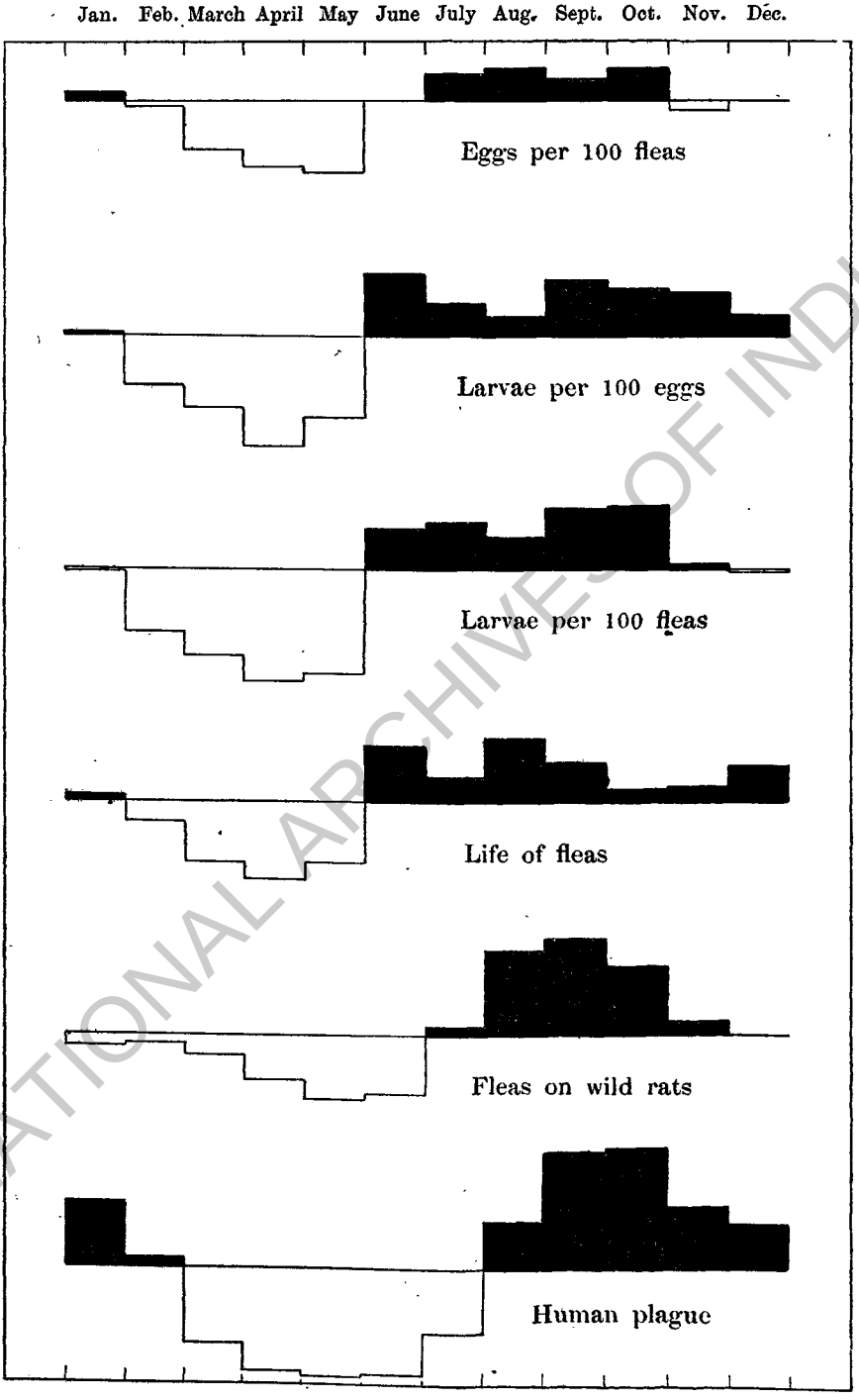

Fig. 7.

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with an average life of 0·8 days giving a total life of 17 minutes or $\frac{1}{4000}$th of the July figure.

These figures cannot of course be transferred directly to the breeding of fleas under natural surroundings. Taken as a whole however they afford strong evidence that the seasonal variation in the prevalence of fleas, which has been shown to be one of the circumstances which condition the occurrence of epidemic plague, is due to factors which exercise a direct influence on the flea, and that among these factors are atmospheric humidity and, to a less extent within the range examined, temperature.

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LVI. THE SERUM TREATMENT OF HUMAN PLAGUE.

THE present series of observations on the treatment of plague with antisera has been carried out at the Maratha plague hospital, Bombay, during the epidemics from 1908—1911. Throughout the work for every case treated with serum a parallel case has been left without serum treatment; all cases received alike the ordinary hospital treatment. At first Dr Choksy, the medical officer in charge of the Maratha hospital, selected those which in his opinion were suitable for observation and the alternate cases received serum. Later on, however, when it became apparent that it was not possible on clinical grounds to differentiate the cases with grave, and as it turned out hopeless, septicaemia, every alternate case coming to hospital received serum, the moribund and those who had almost recovered alone being excluded from consideration.

A few cubic centimetres of blood were aseptically taken from a vein of each selected case. One quarter of a cubic centimetre was spread over the surface of an agar tube, and after incubation for 48 hours the cases could be divided into the following four groups:

(1) blood sterile in $\frac{1}{4}$ c.c. = 0,
(2) 1 to 10 colonies from $\frac{1}{4}$ c.c. = +,
(3) 11 to 100 colonies from $\frac{1}{4}$ cc. = + +,
(4) more than 100 colonies from $\frac{1}{4}$ c.c. = + + +

Similar cultures were taken as far as possible from each patient every day till the patient either died or recovered: in this way the progress of the septicaemia could be observed.

Two kinds of serum were used: (1) the ordinary Yersin serum prepared by the injection of dead and afterwards living bacilli, made by the Lister Institute: (2) antitoxic serum from horses injected with the toxic nucleoproteid of the plague bacillus by Rowland (vol. XI. supplement, p. 11) and MacConkey (*infra*, p. 387) which was of proved efficacy in protecting rats from the injection of living broth cultures of plague bacilli. The serum was given in large doses, generally both intravenously and subcutaneously, sometimes subcutaneously only and

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in a few cases intravenously only. In many cases further doses were given (usually subcutaneously) on succeeding days. In this way, as will be seen from table VI, a number of patients received altogether 500 c.c. of serum and upward.

The data concerning each case are shortly set out in table VI. In all 444 cases were observed, 222 being treated with serum and 222 being taken as controls. Table I summarises the results obtained:

TABLE I.

Summary of cases grouped by the degree of septicaemia.

	Serum			Control				
Septicaemia	Died	Recovered	% died	Died	Recovered	% died	Total	% died
0	22	63	25·9	24	46	34·3	155	29·7
+	35	12	74·5	35	12	74·5	94	74·5
++	15	0	100	24	0	100	39	100
+++	75	0	100	81	0	100	156	100
Total	147	75	66·2 %	164	58	73·9 %	444	70·0 %
	222			222				

From this it appears that, while 70 % of the cases without bacilli in the blood survived, three-quarters of those with a slight degree of septicaemia (+) died and none of the 195 with higher grades (++ and +++) recovered. The latter may therefore be excluded in the comparison between serum cases and controls. The percentage fatality in the + group is exactly the same whether serum was used or not, and it is only in the sterile group that any difference in the result is seen. Here, in the controls 24 out of 70 died or 34 %, while of the similar serum cases only 22 died out of 85 or 26 %. The difference therefore is in favour of the serum by some eight cases per hundred, but it is not large enough with such a small number of cases to indicate any definite influence of serum and does not satisfy the customary statistical test for error due to random sampling.

The next tables (II and III) show the same cases divided into two groups according to whether Yersin or antitoxic serum was used: in each case the controls belonging to the same period are given for comparison and the total controls for each group (which is probably the fairer figure) for comparison are given in table I.

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TABLE II.

Summary of cases treated with Yersin serum with the contemporary controls.

	Serum			Controls			
Septicaemia	Died	Recovered	% died	Died	Recovered	% died	Total
0	14	43	24·6	10	31	24·4	98
+	22	8	73·3	32	10	76·2	72
+ +	12	0	100	16	0	100	28
+ + +	47	0	100	47	0	100	94
Total	95	51	65·1 %	105	41	71·9 %	292
	146			146			

TABLE III.

Summary of cases treated with antitoxic serum with the contemporary controls.

	Serum			Controls			
Septicaemia	Died	Recovered	% died	Died	Recovered	% died	Total
0	8	20	28·6	14	15	48·3	57
+	13	4	76·5	5	2	71·4	24
+ +	3	0	100	6	0	100	9
+ + +	28	0	100	34	0	100	62
Total	52	24	68·4 %	59	17	77·6 %	152
	76			76			

The Yersin series shows no indication of any advantage for the serum cases as against its own controls and the reduction of mortality (10 %) in group 0 as compared with the total controls is of indefinite significance. In the antitoxic series, the mortality is a good deal less (20 %) than in the contemporary controls but the difference is not statistically significant and the exceptionally high mortality among the controls indicates that they are not a fair sample.

In table IV is shown the average duration of illness in the relevant groups as indicated by the length of their stay in hospital. Making

TABLE IV.

Average of days in hospital of cases which recovered.

Septicaemia	Controls	Yersin serum	Antitoxic serum	Total serum
0	45	43	56	47
+	77	56	45	52

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some allowance for the fact that the convalescence of the serum cases is often somewhat retarded by arthritis and other "serum phenomena," it appears that in group + the serum cases get better rather sooner than the controls. Otherwise the figures are negative.

Table V shows in the same way the duration of life in the fatal cases. As is the case with the percentage fatality (see table I), the length of life varies with the degree of septicaemia. In the grave cases (groups ++ and +++) life appears to be prolonged by the use of serum but it is suspicious that this does not seem to be the case in those with no or a slight septicaemia.

TABLE V.

Average length of life after admission to hospital of cases which died.

Septicaemia	0	+	++	+++	Total
Control	7·4 *	5·2	2·9	2·0	3·6 days
Serum	9·2	5·3	5·3	2·7	4·6 days

* 13·1 if case 52, living 146 days, is included.

From the whole enquiry therefore it appears that the administration of the available sera is not a practicable means of bringing about any material diminution in the mortality from plague in India. It may well be that better results would be obtained if the treatment could be commenced within a few hours of the onset of the disease: this however is, in the great majority of cases, impossible in ordinary hospital practice.

We are glad to take this opportunity of expressing our indebtedness to Dr N. H. Choksy, without whose cordial encouragement and assistance this enquiry would have been hardly possible. Dr Choksy's own observations[1] on the serum treatment of plague have extended over many years and he has an unrivalled experience of the matter.

[1] See *British Medical Journal*, 1908, vol. I. p. 1282 and the references there given. The earlier observations in India are summarised by W. B. Bannerman in *Scientific Memoirs*, No. 20, 1905.

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TABLE VI.

I. *Control cases without serum treatment.*

The fatal cases are shown in heavy type.

Serial number	Hospital number	Sex	Age	Date	Day of disease	Days after admission before discharge	Days after admission before death	Septicaemia
2	539	M	12	27. 4. 08	?	35	—	0
4	**542**	**M**	**15**	**27. 4. 08**	**2**	—	**4**	**++**
6	**548**	**M**	**25**	**28. 4. 08**	**2**	—	**3**	**+++**
8	551	F	15	28. 4. 08	2	48	—	0
10	**561**	**M**	**19**	**29. 4. 08**	**4**	—	**1**	**+++**
12	563	M	25	29. 4. 08	2	47	—	0
14	**567**	**M**	**25**	**29. 4. 08**	**2**	—	**4**	**+++**
16	571	M	12	30. 4. 08	3	31	—	0
18	**585**	**M**	**30**	**2. 5. 08**	**2**	—	**6**	**o**
20	588	M	35	3. 5. 08	4	42	—	0
22	**612**	**M**	**20**	**6. 5. 08**	**3**	—	**3**	**++ to +++**
24	615	M	[35]	6. 5. 08	3	52	—	+ to 0
26	**631**	**M**	**30**	**9. 5. 08**	**4**	—	**4**	**o**
28	649	M	16	12. 5. 08	3	34	—	+ to 0
30	**711**	**M**	**16**	**2. 6. 08**	**4**	—	**9**	**o**
32	712	M	20	2. 6. 08	4	36	—	0
34	130	M	30	15. 3. 09	3	116	—	+ to 0
36	**135**	**M**	**30**	**16. 3. 09**	**4**	—	**3**	**++**
38	**138**	**M**	**5**	**16. 3. 09**	**2**	—	**4**	**++**
40	**142**	**M**	**55**	**17. 3. 09**	**5**	—	**3**	**+++**
42	151	M	35	18. 3. 09	3	39	—	0
44	**158**	**M**	**16**	**19. 3. 09**	**2**	—	**4**	**+ to +++**
46	**160**	**M**	**32**	**19. 3. 09**	**3**	—	**6**	**+ to o to +**
48	161	M	30	19. 3. 09	5	68	—	0
50	**165**	**M**	**30**	**20. 3. 09**	**2**	—	**2**	**+++**
52	**167**	**M**	**35**	**20. 3. 09**	**3**	—	**146**	**o**
54	**170**	**M**	**20**	**20. 3. 09**	**1**	—	**3**	**+++**
56	**188**	**M**	**35**	**22. 3. 09**	**3**	—	**5**	**+ to +++**
58	179	F	27	22. 3. 09	1	28	—	0
60	**189**	**M**	**12**	**22. 3. 09**	**2**	—	**2**	**+++**
62	198	M	25	23. 3. 09	1	34	—	0
64	**201**	**M**	**25**	**24. 3. 09**	**4**	—	**4**	**+ to +++**
66	**205**	**M**	**15**	**24. 3. 09**	**2**	—	**7**	**+ to o**
68	**212**	**M**	**30**	**25. 3. 09**	**4**	—	**4**	**+ to o**
70	**218**	**F**	**10**	**26. 3. 09**	**1**	—	**5**	**+ to +++**
72	**225**	**M**	**20**	**27. 3. 09**	**2**	—	**4**	**++ to +++**
74	**237**	**M**	**35**	**29. 3. 09**	**2**	—	**1**	**+++**
76	239	M	30	29. 3. 09	4	42	—	0
78	**241**	**F**	**26**	**29. 3. 09**	**2**	—	**5**	**+ to +++**
80	**247**	**M**	**44**	**30. 3. 09**	**3**	—	**3**	**+ to +++**

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TABLE VI (*contd.*).

Serial number	Hospital number	Sex	Age	Date	Day of disease	Days after admission before discharge	Days after admission before death	Septicaemia
82	**249**	**F**	**35**	**30. 3. 09**	**2**	—	**13**	**+ to 0**
84	**271**	**M**	**55**	**3. 4. 09**	**2**	—	**1**	**+++**
86	275	M	16	4. 4. 09	2	43	—	+ to 0
88	276	F	35	4. 4. 09	3	29	—	0
90	280	M	16	5. 4. 09	2	42	—	0
92	**282**	**F**	**25**	**5. 4. 09**	**3**	—	**2**	**+++**
94	**299**	**M**	**18**	**8. 4. 09**	**3**	—	**1**	**+++**
96	**302**	**M**	**28**	**9. 4. 09**	**2**	—	**3**	**+ to 0**
98	**304**	**M**	**10**	**9. 4. 09**	**2**	—	**1**	**+++**
100	**309**	**M**	**15**	**9. 4. 09**	**3**	—	**3**	**+ to +++**
102	**319**	**M**	**50**	**11. 4. 09**	**3**	—	**4**	**++ to +**
104	**327**	**M**	**16**	**13. 4. 09**	**2**	—	**3**	**+++**
106	**331**	**M**	**28**	**13. 4. 09**	**3**	—	**3**	**+++**
108	**337**	**F**	**30**	**14. 4. 09**	**3**	—	**4**	**+**
110	**344**	**M**	**28**	**16. 4. 09**	**2**	—	**3**	**+++**
112	**347**	**M**	**35**	**16. 4. 09**	**2**	—	**3**	**++**
114	349	F	18	16. 4. 09	2	52	—	0
116	**352**	**M**	**20**	**17. 4. 09**	**2**	—	**2**	**+++**
118	355	M	20	17. 4. 09	4	61	—	0
120	354	F	11	17. 4. 09	2	37	—	0
122	**362**	**M**	**25**	**18. 4. 09**	**2**	—	**5**	**+++**
124	368	F	25	19. 4. 09	2	21	—	0
126	**369**	**M**	**24**	**20. 4. 09**	**7**	—	**1**	**+++**
128	**372**	**M**	**35**	**20. 4. 09**	**2**	—	**5**	**+++**
130	**382**	**M**	**32**	**21. 4. 09**	**3**	—	**3**	**+ to ++**
132	**386**	**M**	**25**	**21. 4. 09**	**2**	—	**4**	**+ to +++**
134	392	F	5	22. 4. 09	4	81	—	+ to 0
136	**398**	**M**	**25**	**23. 4. 09**	**4**	—	**4**	**+++**
138	**406**	**F**	**9**	**23. 4. 09**	**4**	—	**3**	**+++**
140	**408**	**M**	**40**	**23. 4. 09**	**4**	—	**1**	**+++**
142	**409**	**M**	**60**	**24. 4. 09**	**3**	—	**1**	**+++**
144	**412**	**M**	**24**	**24. 4. 09**	**2**	—	**5**	**0**
146	**415**	**M**	**50**	**25. 4. 09**	**2**	—	**5**	**+ to +++**
148	**423**	**M**	**35**	**26. 4. 09**	**3**	—	**3**	**+++**
150	**428**	**M**	**12**	**26. 4. 09**	**2**	—	**3**	**+++**
152	429	M	17	27. 4. 09	4	50	—	+ to 0
154	**446**	**F**	**30**	**30. 4. 09**	**2**	—	**3**	**+++**
156	**448**	**M**	**23**	**30. 4. 09**	**2**	—	**1**	**++**
158	**469**	**M**	**19**	**2. 5. 09**	**1**	—	**4**	**+++**
160	**477**	**M**	**45**	**4. 5. 09**	**2**	—	**3**	**+++**
162	**481**	**M**	**20**	**5. 5. 09**	**3**	—	**4**	**+ to +++**
164	498	F	32	10. 5. 09	7	84	—	0
166	**84**	**M**	**35**	**15. 3. 10**	**2**	—	**3**	**+++**
168	91	M	30	16. 3. 10	4	53	—	0
170	**94**	**M**	**20**	**16. 3. 10**	**2**	—	**1**	**+++**

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TABLE VI (*contd.*).

Serial number	Hospital number	Sex	Age	Date	Day of disease	Days after admission before discharge	Days after admission before death	Septicaemia
172	98	M	25	18. 3. 10	4	—	3	+
174	102	M	11	20. 3. 10	4	—	7	+ to +++
176	105	M	30	20. 3. 10	2	—	2	++ to +++
178	110	M	40	23. 3. 10	3	—	3	+ to 0
180	112	M	14	23. 3. 10	4	—	3	+++
182	116	M	20	24. 3. 10	5	—	2	+++
184	120	M	28	26. 3. 10	2	51	—	0
186	126	M	35	30. 3. 10	4	—	2	+ to ++
188	129	M	25	30. 3. 10	2	—	2	+++
190	131	M	25	31. 3. 10	4	—	6	0
192	134	M	14	31. 3. 10	4	—	1	++
194	137	M	30	2. 4. 10	2	—	1	+++
196	144	M	18	5. 4. 10	4	—	1	++ to +++
198	153	M	25	7. 4. 10	5	108	—	0
200	168	M	30	13. 4. 10	3	—	1	+++
202	172	M	25	15. 4. 10	4	—	2	+ to ++
204	180	M	18	17. 4. 10	4	109	—	+ to 0
206	186	M	18	20. 4. 10	3	—	2	+++
208	191	M	14	21. 4. 10	2	15	—	0
210	197	M	30	22. 4. 10	6	59	—	0
212	199	M	40	22. 4. 10	4	46	—	+ to 0
214	205	M	45	25. 4. 10	2	—	7	+ to 0
216	210	M	50	27. 4. 10	3	26	—	0
218	216	F	35	28. 4. 10	4	—	2	++ to +++
220	227	M	20	30. 4. 10	2	—	2	+++
222	237	M	20	3. 5. 10	3	—	2	+++
224	243	M	40	3. 5. 10	2	62	—	0
226	246	F	35	5. 5. 10	5	—	2	+++
228	256	M	24	6. 5. 10	2	—	5	+ to 0
230	275	F	30	11. 5. 10	4	—	3	+++
232	277	M	35	12. 5. 10	4	—	6	0
234	290	M	30	13. 5. 10	3	—	4	+ to 0
236	294	M	25	14. 5. 10	5	73	—	0
238	19	M	22	30. 1. 11	3	—	1	+++
240	22	F	11	2. 2. 11	4	—	1	+++
242	25	M	30	6. 2. 11	4	—	4	0
244	30	M	20	8. 2. 11	4	—	7	+ to 0 to +
246	32	M	15	9. 2. 11	4	—	3	++
248	34	M	35	10. 2. 11	4	26	—	0
250	36	M	20	11. 2. 11	4	—	1	+++
252	43	M	30	16. 2. 11	4	61	—	+ to 0 to + to 0
254	49	F	35	19. 2. 11	4	—	13	0
256	54	M	20	20. 2. 11	4	—	3	+++
258	67	M	17	28. 2. 11	3	—	4	+ to +++
260	71	M	45	1. 3. 11	3	—	1	0

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TABLE VI. (*contd.*).

Serial number	Hospital number	Sex	Age	Date	Day of disease	Days after admission before discharge	Days after admission before death	Septicaemia
262	**84**	**M**	**25**	**6. 3. 11**	**5**	—	**5**	**O**
264	**90**	**M**	**25**	**7. 3. 11**	**2**	—	**1**	**O**
266	92	M	30	8. 3. 11	5	68	—	0
268	**93**	**M**	**17**	**7. 3. 11**	**3**	—	**1**	**+++**
270	**99**	**M**	**13**	**10. 3. 11**	**3**	—	**1**	**+++**
272	**105**	**M**	**19**	**11. 3. 11**	**5**	—	**3**	**++ to +++**
274	107	F	45	11. 3. 11	5	45	—	0
276	**110**	**M**	**20**	**13. 3. 11**	**3**	—	**2**	**+++**
278	112	F	20	13. 3. 11	3	49	—	0
280	118	F	8	17. 3. 11	3	39	—	0
282	**124**	**M**	**35**	**19. 3. 11**	**4**	—	**4**	**O**
284	**128**	**M**	**58**	**20. 3. 11**	**3**	—	**4**	**O**
286	**136**	**M**	**30**	**21. 3. 11**	**4**	—	**3**	**+++**
288	**137**	**M**	**30**	**21. 3. 11**	**3**	—	**1**	**+++**
290	**147**	**M**	**22**	**23. 3. 11**	**2**	—	**3**	**+++**
292	**150**	**M**	**50**	**24. 3. 11**	**3**	—	**4**	**++ to +++**
294	157	M	30	25. 3. 11	3	51	—	0
296	**161**	**M**	**16**	**27. 3. 11**	**3**	—	**1**	**+++**
298	165	M	30	28. 3. 11	3	48	—	0
300	169	M	35	28. 3. 11	5	34	—	0
302	**173**	**M**	**28**	**29. 3. 11**	**5**	—	**3**	**++**
304	**178**	**M**	**30**	**30. 3. 11**	**2**	—	**1**	**+++**
306	**180**	**M**	**22**	**30. 3. 11**	**3**	—	**6**	**O**
308	**187**	**M**	**25**	**31. 3. 11**	**3**	—	**1**	**+++**
310	196	M	25	2. 4. 11	2	29	—	0
312	204	F	40	3. 4. 11	2	28	—	0
314	**207**	**M**	**18**	**4. 4. 11**	**4**	—	**3**	**+++**
316	**209**	**M**	**25**	**4. 4. 11**	**4**	—	**1**	**+++**
318	218	F	20	5. 4. 11	4	33	—	0
320	220	M	16	5. 4. 11	6	20	—	0
322	**222**	**M**	**22**	**5. 4. 11**	**4**	—	**8**	**+ to O**
324	**226**	**M**	**30**	**5. 4. 11**	**4**	—	**2**	**+++**
326	**230**	**M**	**30**	**6. 4. 11**	**4**	—	**4**	**++**
328	235	F	12	7. 4. 11	3	38	—	+ to 0
330	**247**	**M**	**50**	**8. 4. 11**	**5**	—	**3**	**O**
332	**252**	**M**	**60**	**8. 4. 11**	**2**	—	**5**	**++**
334	**254**	**M**	**40**	**9. 4. 11**	**3**	—	**4**	**+++**
336	**257**	**M**	**16**	**9. 4. 11**	**4**	—	**4**	**+++**
338	**264**	**F**	**18**	**10. 4. 11**	**3**	—	**9**	**O to +**
340	**260**	**M**	**30**	**10. 4. 11**	**3**	—	**2**	**+++**
342	**268**	**M**	**28**	**11. 4. 11**	**5**	—	**1**	**+++**
344	**271**	**M**	**35**	**11. 4. 11**	**2**	—	**1**	**+++**
346	**272**	**F**	**45**	**12. 4. 11**	**3**	—	**1**	**+++**
348	**275**	**M**	**25**	**12. 4. 11**	**5**	—	**16**	**O**
350	**277**	**M**	**12**	**12. 4. 11**	**3**	—	**8**	**O**

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TABLE VI (*contd.*).

Serial number	Hospital number	Sex	Age	Date	Day of disease	Days after admission before discharge	Days after admission before death	Septicaemia
352	280	M	30	12. 4. 11	4	—	8	O
354	283	M	30	12. 4. 11	2	—	2	+++
356	290	F	12	13. 4. 11	5	32	—	0
358	298	M	30	15. 4. 11	3	—	3	+++
360	300	M	22	15. 4. 11	3	—	3	+++
362	304	M	55	16. 4. 11	2	—	1	+++
364	309	M	30	17. 4. 11	5	—	1	+++
366	315	M	20	18. 4 11	3	—	1	+++
368	319	M	22	18. 4. 11	3	—	4	+++
370	320	M	40	18. 4. 11	5	—	1	+++
372	322	M	25	20. 4. 11	3	—	5	+ to +++
374	324	M	60	19. 4. 11	3	—	3	+++
376	329	M	25	20. 4. 11	5	61	—	0
378	333	M	30	21. 4. 11	5	72	—	0
380	335	F	14	21. 4. 11	4	—	5	+ to O to +
382	337	M	45	21. 4. 11	4	—	1	+++
384	342	M	22	22. 4. 11	5	—	1	+++
386	344	M	20	22. 4. 11	5	—	6	O
388	347	M	25	22. 4. 11	4	—	1	+++
390	350	F	30	24. 4. 11	4	—	4	O
392	356	F	22	25. 4. 11	3	—	7	+ to O
394	358	M	20	27. 4. 11	5	—	1	O
396	367	M	25	27. 4. 11	2	—	10	+ to O
398	369	F	20	27. 4. 11	2	43	—	0
400	372	M	30	28. 4. 11	3	—	2	+++
402	376	M	10	28. 4. 11	2	—	1	+++
404	381	M	30	28. 4. 11	2	64	—	0
406	385	M	22	29. 4. 11	3	—	3	++
408	387	M	40	29. 4. 11	3	—	1	+++
410	394	M	22	1. 5. 11	3	—	1	++
412	401	M	30	3. 5. 11	4	70	—	0
414	409	M	22	4. 5. 11	4	—	1	+++
416	411	M	18	4. 5. 11	3	—	2	+++
418	417	M	40	6. 5. 11	3	—	1	+++
420	422	M	38	7. 5. 11	5	—	4	++
422	423	M	16	8. 5. 11	5	—	40	O
424	432	M	22	8. 5. 11	2	—	5	+
426	438	M	14	10. 5. 11	2	—	4	++ to + to ++
428	442	F	14	11. 5. 11	3	—	4	+++
430	445	M	12	13. 5. 11	3	23	—	0
432	447	M	12	14. 5. 11	3	172	—	+ to 0
434	460	M	35	16. 5. 11	6	—	1	++
436	462	F	35	16. 5. 11	5	—	5	+ to O to +
438	467	M	25	17. 5. 11	3	—	3	++
440	470	F	18	22. 5. 11	3	90	—	+ to 0
442	476	M	20	24. 5. 11	2	—	13	+ to O
444	479	M	12	25. 5. 11	6	68	—	+ to 0

NATIONAL ARCHIVES OF INDIA

TABLE VI (*contd.*).

II. *Cases treated with serum.*

A. Treated with Yersin's serum.

Serial number	Hospital number	Sex	Age	Date	Day of disease	Days after treatment before discharge	Days after treatment before death	Septicaemia	Serum given c.c. subcut.	Serum given c.c. intraven
1	540	M	25	27. 4. 08	3	35	—	0	—	180
3	**541**	**M**	**40**	**27. 4. 08**	**1**	—	**2**	**+++**	—	**100**
5	**547**	**M**	**25**	**28. 4. 08**	**1**	—	**3**	**++**	—	**95**
7	554	F	40	28. 4. 08	4	35	—	0	—	100
9	**557**	**M**	**13**	**29. 4. 08**	**2**	—	**3**	**+++**	—	**100**
11	**560**	**M**	**20**	**29. 4. 08**	**4**	—	**3**	**++**	—	**150**
13	**568**	**M**	**20**	**29. 4. 08**	**1**	—	**3**	**+++**	—	**50**
15	**569**	**M**	**30**	**29. 4. 08**	**2**	—	**8**	**+ to 0**	—	**610**
17	**572**	**F**	**20**	**30. 4. 08**	**3**	—	**4**	**++**	—	**150**
19	**586**	**M**	**22**	**3. 5. 08**	**3**	—	**3**	**++ to +++**	—	**200**
21	589	M	15	3. 5. 08	2	43	—	+ to 0	100	400
23	**613**	**M**	**29**	**6. 5. 08**	**4**	—	**7**	**0**	**320**	**400**
25	632	F	55	9. 5. 08	4	48	—	0	160	300
27	**637**	**M**	**40**	**9. 5. 08**	**2**	—	**25**	**0**	**220**	**200**
29	713	M	16	2. 6. 08	2	42	—	0	90	100
31	**714**	**M**	**40**	**3. 6. 08**	**3**	—	**1**	**+++**	**100**	**100**
33	133	M	23	15. 3. 09	4	77	—	0	320	—
35	136	M	30	16. 3. 09	2	69	—	+ to 0	220	210
37	141	M	20	17. 3. 09	6	27	—	0	100	—
39	**146**	**M**	**30**	**18. 3. 09**	**4**	—	**3**	**+++**	**160**	**170**
41	150	M	35	18. 3. 09	2	39	—	0	220	—
43	152	F	45	18. 3. 09	2	52	—	0	360	—
45	**155**	**M**	**55**	**18. 3. 09**	**1**	—	**6**	**++ to + to ++**	**220**	**140**
47	162	M	22	19. 3. 09	2	52	—	+ to 0	320	220
49	**164**	**M**	**18**	**20. 3. 09**	**3**	—	**2**	**+++**	**160**	**160**
51	166	M	18	20. 3. 09	2	44	—	0	280	280
53	**169**	**F**	**23**	**20. 3. 09**	**3**	—	**3**	**+++**	**160**	**120**
55	**173**	**M**	**30**	**21. 3. 09**	**3**	—	**1**	**+++**	**160**	**170**
57	183	M	20	22. 3. 09	1	56	—	0	520	180
59	**186**	**M**	**30**	**22. 3. 09**	**3**	—	**4**	**+**	**220**	**220**
61	192	F	15	22. 3. 09	1	37	—	0	360	160
63	**200**	**M**	**25**	**24. 3. 09**	**5**	—	**11**	**0**	**500**	**100**
65	**202**	**M**	**16**	**24. 3. 09**	**2**	—	**8**	**++ to 0 to ++**	**600**	—
67	**206**	**M**	**57**	**25. 3. 09**	**4**	—	**3**	**+++ to ++**	**220**	**160**
69	210	M	6	25. 3. 09	1	35	—	0	280	—
71	**219**	**M**	**12**	**27. 3. 09**	**3**	—	**11**	**+ to 0 to +**	**560**	—
73	**231**	**F**	**12**	**28. 3. 09**	**3**	—	**2**	**0**	**100**	**100**
75	238	M	20	29. 3. 09	4	41	—	0	260	100
77	**245**	**M**	**12**	**30. 3. 09**	**4**	—	**1**	**+++**	**100**	**100**
79	**246**	**F**	**25**	**30. 3. 09**	**3**	—	**30**	**+ to 0**	**580**	**220**

NATIONAL ARCHIVES OF INDIA

TABLE VI (*contd.*).

Serial number	Hospital number	Sex	Age	Date	Day of disease	Days after treatment before discharge	Days after treatment before death	Septicaemia	Serum given c.c. subcut.	Serum given c.c. intraven.
81	254	M	40	31. 3. 09	2	33	—	0	220	160
83	258	M	25	1. 4. 09	3	39	—	0	160	80
85	277	F	20	4. 4. 09	4	—	6	+++ to 0 to +	500	—
87	278	M	25	4. 4. 09	1	43	—	0	320	165
89	279	M	30	4. 4. 09	5	—	1	0	100	100
91	281	M	30	5. 4. 09	3	—	3	+++	100	100
93	298	M	50	8. 4. 09	3	39	—	+ to 0	280	120
95	306	M	18	9. 4. 09	5	45	—	0	220	140
97	307	M	21	9. 4. 09	3	39	—	0	380	280
99	308	M	25	9. 4. 09	2	68	—	+ to 0	280	280
101	318	M	15	11. 4. 09	2	36	—	0	220	160
103	322	F	28	11. 4. 09	2	—	2	+++	200	—
105	325	F	28	13. 4. 09	3	—	7	+++ to + to +++	160	560
107	328	M	30	13. 4. 09	2	—	2	+++	100	100
109	340	M	39	15. 4. 09	2	—	3	+++	100	100
111	345	M	26	16. 4. 09	2	31	—	0	160	160
113	350	F	15	16. 4. 09	1	24	—	0	140	80
115	351	M	30	16. 4. 09	2	—	1	+++	100	100
117	353	M	15	17. 4. 09	2	—	3	+++ to 0	360	120
119	357	M	20	18. 4. 09	3	—	3	+++	200	60
121	359	M	30	18. 4. 09	5	—	3	++ to +	240	160
123	364	M	24	19. 4. 09	2	35	—	0	300	100
125	367	F	19	19. 4. 09	2	—	1	+	100	100
127	371	M	18	20. 4. 09	3	—	9	+ to 0	320	220
129	374	M	30	20. 4. 09	1	33	—	0	360	100
131	390	M	12	22. 4. 09	4	—	1	+++	60	40
133	394	F	24	22. 4. 09	3	—	4	+++	220	220
135	395	M	35	22. 4. 09	2	—	2	0	100	100
137	399	M	42	23. 4. 09	1	—	8	++ to 0	420	220
139	401	M	30	23. 4. 09	3	—	5	++ to + to ++	320	220
141	402	F	22	23. 4. 09	2	—	3	0	100	100
143	411	M	14	24. 4. 09	3	—	5	+++ to + to ++	140	100
145	414	M	32	25. 4. 09	3	—	4	+++ to ++ to +	160	140
147	422	M	22	26. 4. 09	3	—	6	+++ to +	160	120
149	424	F	9	26. 4. 09	2	—	3	+++	60	60
151	430	M	30	27. 4. 09	2	—	12	+++ to 0 to +	200	60
153	436	F	30	28. 4. 09	2	—	6	+++ to + to +++	160	120
155	440	F	40	29. 4. 09	3	—	4	0 to + to 0	300	—
157	447	F	14	30. 4. 09	2	—	1	0	60	60
159	462	F	25	2. 5. 09	2	—	6	+++ to +	100	60
161	465	M	32	2. 5. 09	3	—	1	+++	100	60
163	483	M	65	5. 5. 09	3	—	5	0	320	—
165	83	M	25	15. 3. 10	5	—	4	+ to 0	400	150
167	87	M	30	15. 3. 10	4	75	—	+ to 0	550	200
169	92	M	38	16. 3. 10	4	—	6	+ to 0	450	115
171	97	F	7	18. 3. 10	2	51	—	0	180	—

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TABLE VI (*contd.*).

Serial number	Hospital number	Sex	Age	Date	Day of disease	Days after treatment before discharge	Days after treatment before death	Septicaemia	Serum given c.c. subcut.	Serum given c.c. intraven.
173	**99**	**M**	**25**	**18. 3. 10**	**3**	—	**5**	**+++ to ++**	**300**	**150**
175	**101**	**M**	**16**	**18. 3. 10**	**3**	—	**4**	**+++ to ++**	**400**	**100**
177	**107**	**M**	**35**	**20. 3. 10**	**4**	—	**1**	**+++**	**200**	**100**
179	**111**	**M**	**45**	**22. 3. 10**	**3**	—	**2**	**+++**	**100**	**50**
181	**114**	**M**	**20**	**23. 3. 10**	**5**	—	**4**	**+++**	**300**	**150**
183	**118**	**M**	**30**	**24. 3. 10**	**3**	—	**1**	**+++**	**150**	—
185	127	M	28	30. 3. 10	4	40	—	0	520	150
187	128	M	16	30. 3. 10	2	40	—	0	520	50
189	**132**	**M**	**28**	**31. 3. 10**	**3**	—	**7**	**+ to O**	**580**	**150**
191	**133**	**M**	**25**	**31. 3. 10**	**4**	—	**5**	**+++ to +**	**300**	**150**
193	**136**	**M**	**30**	**1. 4. 10**	**2**	—	**1**	**+++**	**300**	**150**
195	**143**	**M**	**25**	**3. 4. 10**	**3**	—	**3**	**+++**	**300**	**150**
197	151	M	10	5. 4. 10	1	22	—	0	100	50
199	167	M	30	13. 4. 10	4	42	—	0	200	100
201	**171**	**M**	**18**	**15. 4. 10**	**4**	—	**4**	**+ to ++**	**400**	**150**
203	**178**	**M**	**44**	**16. 4. 10**	**2**	—	**11**	**+ to O**	**720**	**45**
205	184	M	20	19. 4. 10	3	62	—	0	500	150
207	190	M	20	20. 4. 10	4	41	—	0	400	150
209	193	M	35	21. 4. 10	2	39	—	+ to 0	400	150
211	**198**	**M**	**35**	**22. 4. 10**	**3**	—	**2**	**+++**	**300**	**150**
213	204	M	30	25. 4. 10	3	92	—	0	500	200
215	**207**	**M**	**18**	**26. 4. 10**	**2**	—	**7**	**+ to O**	**560**	**150**
217	**212**	**M**	**30**	**27. 4. 10**	**2**	—	**7**	**+ to O to ++**	**560**	**150**
219	222	M	20	30. 4. 10	2	21	—	0	460	150
221	**235**	**M**	**16**	**3. 5. 10**	**5**	—	**6**	**+ to O**	**300**	**150**
223	242	M	15	3. 5. 10	1	34	—	0	400	150
225	244	F	35	5. 5. 10	5	47	—	0	460	150
227	**255**	**M**	**16**	**6. 5. 10**	**3**	—	**1**	**+++**	**300**	—
229	**268**	**F**	**27**	**10. 5. 10**	**3**	—	**1**	**O**	**200**	**100**
231	282	M	28	11. 5. 10	3	26	—	0	300	150
233	**291**	**M**	**25**	**14. 5. 10**	**4**	—	**3**	**+ to O**	**500**	**25**
235	289	M	25	13. 5. 10	3	54	—	0	450	—
387	**343**	**M**	**20**	**22. 4. 11**	**5**	—	**2**	**+++**	**200**	**80**
391	**349**	**M**	**20**	**23. 4. 11**	**4**	—	**1**	**+++**	**100**	**40**
393	355	M	15	25. 4. 11	4	35	—	0	400	120
395	362	M	58	26. 4. 11	3	54	—	0	400	80
397	**366**	**M**	**15**	**27. 4. 11**	**4**	—	**16**	**O**	**400**	**80**
399	**368**	**M**	**20**	**27. 4. 11**	**2**	—	**1**	**+**	**100**	**40**
401	**370**	**M**	**22**	**28. 4. 11**	**3**	—	**3**	**+++**	**200**	**80**
403	**374**	**F**	**30**	**28. 4. 11**	**4**	—	**7**	**+ to O**	**560**	**80**
405	**380**	**M**	**22**	**28. 4. 11**	**4**	—	**46**	**O**	**400**	**80**
407	**382**	**M**	**32**	**28. 4. 11**	**3**	—	**3**	**+++**	**200**	**80**
409	386	M	33	29. 4. 11	4	44	—	0	600	80
411	**391**	**M**	**29**	**29. 4. 11**	**3**	—	**3**	**+ to O**	**200**	**80**
413	**396**	**M**	**12**	**1. 5. 11**	**3**	—	**7**	**++ to O to +**	**500**	**20**
415	**406**	**M**	**38**	**4. 5. 11**	**4**	—	**1**	**O**	**100**	**40**

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TABLE VI (*contd.*).

Serial number	Hospital number	Sex	Age	Date	Day of disease	Days after treatment before discharge	Days after treatment before death	Septicaemia	Serum given c.c. subcut.	Serum given c.c. intraven.
417	**414**	**M**	**18**	**5. 5. 11**	**3**	—	**1**	**+++**	**100**	**40**
419	**421**	**M**	**30**	**7. 5. 11**	**4**	—	**1**	**+++**	**100**	**40**
421	427	M	30	7. 5. 11	5	55	—	0	400	80
423	**428**	**M**	**24**	**7. 5. 11**	**2**	—	**5**	**++ to +**	**400**	**80**
425	439	M	20	10. 5. 11	2	68	—	0	260	80
427	444	M	32	12. 5. 11	3	66	—	0	300	80
429	446	M	30	13. 5. 11	6	60	—	+ to 0	500	120
431	**449**	**M**	**14**	**14. 5. 11**	**2**	—	**3**	**++**	**300**	**80**
433	**461**	**M**	**20**	**16. 5. 11**	**4**	—	**2**	**+**	**200**	**80**
435	**463**	**F**	**25**	**16. 5. 11**	**5**	—	**19**	**+ to 0**	**500**	**100**
437	468	M	30	19. 5. 11	3	44	—	0	300	80
439	**471**	**M**	**45**	**22. 5. 11**	**3**	—	**4**	**+**	**260**	**40**
441	**477**	**M**	**21**	**24. 5. 11**	**3**	—	**2**	**+++**	**200**	**80**
443	**485**	**M**	**30**	**28. 5. 11**	**3**	—	**1**	**+**	**100**	**40**

B. Treated with antitoxic serum.

237	**8**	**M**	**16**	**21. 1. 11**	**3**	—	**1**	**0**	**100**	**25**
239	20	M	27	30. 1. 11	1	57	—	0	240	40
241	23	F	53	2. 2. 11	5	91	—	0	180	40
243	27	F	16	7. 2. 11	4	67	—	0	240	—
245	**29**	**F**	**20**	**7. 2. 11**	**4**	—	**4**	**++ to 0 to +**	**240**	**60**
247	31	M	18	8. 2. 11	4	76	—	0	240	—
249	**33**	**F**	**45**	**9. 2. 11**	**6**	—	**8**	**+ to 0 to ++**	**100**	**40**
251	35	M	30	10. 2. 11	4	66	—	0	120	40
253	37	F	35	11. 2. 11	3	72	—	0	300	40
255	44	F	30	16. 2. 11	4	61	—	0	380	100
257	65	M	40	27. 2. 11	4	63	—	0	400	80
259	**70**	**M**	**30**	**1. 3. 11**	**6**	—	**4**	**+ to +++**	**300**	**120**
261	72	F	21	1. 3. 11	2	34	—	+ to 0	500	120
263	**87**	**M**	**16**	**6. 3. 11**	**2**	—	**8**	**0**	**600**	**120**
265	**91**	**F**	**30**	**7. 3. 11**	**4**	—	**10**	**0**	**660**	**120**
267	94	M	12	8. 3. 11	3	68	—	0	560	120
269	**97**	**M**	**18**	**9. 3. 11**	**4**	—	**4**	**++ to +++**	**400**	**80**
271	**101**	**M**	**35**	**10. 3. 11**	**4**	—	**1**	**+++**	**100**	**40**
273	**106**	**M**	**22**	**11. 3. 11**	**2**	—	**10**	**0**	**460**	**80**
275	**108**	**F**	**30**	**12. 3. 11**	**3**	—	**1**	**+++**	**100**	**40**
277	**111**	**F**	**40**	**13. 3. 11**	**2**	—	**9**	**0**	**400**	**120**
279	113	M	23	13. 3. 11	3	68	—	0	460	80
281	**122**	**M**	**35**	**19. 3. 11**	**4**	—	**1**	**+++**	**100**	**40**
283	**127**	**M**	**20**	**19. 3. 11**	**3**	—	**4**	**+ to 0 to +**	**300**	**40**
285	**132**	**M**	**30**	**20. 3. 11**	**5**	—	**3**	**+++**	**200**	**40**
287	**134**	**M**	**29**	**20. 3. 11**	**3**	—	**4**	**+ to 0 to ++**	**400**	**100**
289	**144**	**M**	**24**	**22. 3. 11**	**3**	—	**5**	**+++**	**400**	**100**
291	**148**	**M**	**35**	**23. 3. 11**	**2**	—	**4**	**+++**	**300**	**120**
293	151	M	30	24. 3. 11	5	59	—	0	260	80
295	**159**	**M**	**20**	**26. 3. 11**	**5**	—	**4**	**+++**	**300**	**100**

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TABLE VI (*contd.*).

Serial number	Hospital number	Sex	Age	Date	Day of disease	Days after treatment before discharge	Days after treatment before death	Septicaemia	Serum given c.c. subcut.	Serum given c.c. intraven.
297	**168**	**M**	**17**	**27. 3. 11**	**3**	—	**5**	**+++**	**400**	**120**
299	**168**	**M**	**30**	**28. 3. 11**	**3**	—	**1**	**+++**	**100**	**40**
301	170	F	20	28. 3. 11	3	34	—	0	400	40
303	**172**	**F**	**22**	**28. 3. 11**	**2**	—	**1**	**+++**	**100**	**40**
305	**176**	**F**	**4**	**29. 3. 11**	**3**	—	**1**	**+++**	**40**	—
307	**185**	**M**	**20**	**31. 3. 11**	**2**	—	**5**	**+ to 0**	**300**	**120**
309	189	F	50	31. 3. 11	3	18	—	0	300	80
311	**195**	**M**	**20**	**31. 3. 11**	**2**	—	**1**	**+**	**100**	**40**
313	205	M	30	4. 4. 11	3	51	—	0	500	120
315	**208**	**M**	**50**	**4. 4. 11**	**5**	—	**8**	**+**	**500**	**120**
317	**210**	**M**	**16**	**4. 4. 11**	**3**	—	**1**	**+++**	**100**	**40**
319	**214**	**M**	**30**	**5. 4. 11**	**3**	—	**5**	**+ to ++**	**400**	**120**
321	**219**	**F**	**25**	**5. 4. 11**	**5**	—	**3**	**+++**	**200**	**80**
323	**221**	**M**	**26**	**5. 4. 11**	**2**	—	**1**	**+**	**100**	**40**
325	223	M	15	5. 4. 11	5	55	—	+ to 0	600	80
327	**229**	**M**	**24**	**6. 4. 11**	**1**	—	**6**	**0**	**500**	**120**
329	234	M	15	7. 4. 11	4	38	—	0	400	120
331	**238**	**F**	**22**	**7. 4. 11**	**3**	—	**1**	**+++**	**100**	**40**
333	243	M	14	7. 4. 11	4	73	—	0	400	120
335	**251**	**M**	**25**	**8. 4. 11**	**4**	—	**1**	**+++**	**100**	**40**
337	**258**	**M**	**12**	**9. 4. 11**	**3**	—	**1**	**+++**	**100**	**40**
339	**256**	**M**	**20**	**9. 4. 11**	**3**	—	**4**	**+++**	**400**	**120**
341	261	M	30	10. 4. 11	2	57	—	0	400	120
343	266	M	30	10. 4. 11	1	19	—	0	260	80
345	**265**	**F**	**40**	**10. 4. 11**	**3**	—	**5**	**+**	**300**	**120**
347	262	M	28	10. 4. 11	4	30	—	0	300	80
349	**267**	**M**	**33**	**10. 4. 11**	**2**	—	**1**	**+++**	**200**	**80**
351	**273**	**M**	**30**	**12. 4. 11**	**3**	—	**2**	**+++**	**100**	**40**
353	276	M	15	12. 4. 11	3	55	—	0	400	120
355	**297**	**F**	**31**	**12. 4. 11**	**3**	—	**11**	**0**	**500**	**80**
357	**282**	**M**	**15**	**12. 4. 11**	**5**	—	**4**	**+ to 0**	**400**	**80**
359	**284**	**M**	**23**	**13. 4. 11**	**3**	—	**1**	**+++**	**100**	**40**
361	**294**	**M**	**23**	**14. 4. 11**	**3**	—	**3**	**+++**	**200**	**80**
363	**299**	**M**	**25**	**15. 4. 11**	**3**	—	**4**	**+++**	**300**	**80**
365	**302**	**M**	**52**	**16. 4. 11**	**6**	—	**4**	**+ to 0**	**360**	**80**
367	**306**	**M**	**17**	**16. 4. 11**	**3**	—	**4**	**+++**	**200**	**80**
369	**312**	**M**	**22**	**17. 4. 11**	**4**	—	**1**	**++**	**200**	**80**
371	**316**	**M**	**30**	**18. 4. 11**	**3**	—	**1**	**+++**	**100**	**40**
373	**318**	**M**	**57**	**18. 4. 11**	**4**	—	**4**	**+**	**300**	—
375	323	M	22	19. 4. 11	3	48	—	+ to 0	300	80
377	328	M	30	20. 4. 11	4	44	—	+ to 0	400	40
379	**331**	**M**	**22**	**20. 4. 11**	**2**	—	**1**	**+++**	**200**	**40**
381	**332**	**M**	**25**	**20. 4. 11**	**5**	—	**1**	**+++**	**100**	**40**
383	**338**	**M**	**40**	**21. 4. 11**	**5**	—	**1**	**+++**	**100**	**40**
385	**341**	**M**	**20**	**21. 4. 11**	**3**	—	**19**	**0**	**500**	**40**
389	**345**	**M**	**20**	**22. 4. 11**	**3**	—	**1**	**+++**	**100**	**40**

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LVII. ATTEMPT TO SEPARATE THE ANTIGEN FROM THE NUCLEOPROTEIN OF THE PLAGUE BACILLUS BY FILTRATION THROUGH GELATIN.

By SYDNEY ROWLAND, M.A., M.R.C.S.

Of the Lister Institute.

The antigen of plague as we have established in the course of these reports is intimately associated with a nucleoprotein soluble in dilute salines. By extracting this nucleoprotein from the bacillus the contained antigen (for rats) can be obtained. The relations that exist between the amount of antigen contained in the bacillus and the amount of antigen associated with the nucleoprotein extractable from the bacillus show that in the process of extraction there is little or no loss. The process is, within the limits of experimental error imposed on all measurements based on animal experiments, a quantitative one. In this respect it differs from the process of Lustig and Galeotti[1]. These observers used a 0·75% solution of NaOH as an extraction medium and the quantity of nucleoprotein obtained by their method necessary to immunise a rat is given by them as 0·36 mg. If this amount be compared with the amount of nucleoprotein associated with one protecting dose of the extract obtained by the sulphate of soda method, a striking contrast is apparent. A dose of extract containing 0·0001 mg. protects about 60 per cent. of the rats which receive it (*Journal of Hygiene*, vol. x. (1910), p. 559).

Not only is the associated quantity of nucleoprotein much smaller when the sulphate process is employed, but as far as can be seen there is no loss of antigen in the process. Thus the relations by weight of the nucleoprotein to the bacillus (both weighed dry) are as about ten to one, *i.e.*, from one gram of dry bacilli about 0·1 gram of nucleoprotein is obtained. The protective values of the bacillus and the nucleoprotein obtained from it are about the same. Thus the

[1] *Deutsch. med. Woch.* 1897, pp. 23, 227, 289.

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mortality after the injection of rats with 0·03 mg. of whole bacillus is about 25 %, while the mortality after injecting 0·001 mg. nucleoprotein is about 12 %, the mortalities in both cases occurring after the injection into the test animals of the same quantity of the same virulent broth culture. It is very difficult to obtain exact experimental proof that no loss of antigen occurs on extraction; the figures just quoted show that there is no appreciable loss. In 0·03 mg. of whole bacillus there is 0·003 mg. of nucleoprotein. Inoculation of one-third of this amount was followed by a better immunity than followed the inoculation of 0·03 mg. of whole bacillus.

The question of the relations that exist between the nucleoprotein and the antigen might be elucidated by a study of the effects of filtering the nucleoprotein solution through gelatin, as the gelatin filter serves as a ready means of removing proteins from their solutions. Thus it would be possible to ascertain whether the antigen was so closely bound to the nucleoprotein that gelatin might or might not effect a separation more or less complete between them. More especially was this thought to be worth investigating in view of the results obtained by hydrolysing the nucleoprotein.

Hydrolysis was found to affect the nucleoprotein whilst leaving the antigen intact (vol. XI. (1911), supplement, p. 35). It was possible, therefore, that one of the effects of hydrolysis was to effect a separation between the antigen and the nucleoprotein. The data required are (1) the nucleoprotein content and antigenic value of a solution: (2) the same figures with regard to the same solution after passing through gelatin. With a view to obtaining information on this question the following experiments were carried out.

A solution was prepared. Its antigenic value is given by the experiments in the following table:

Dose	No. rats	No. survived	% survived
0·01 mg.	14	12	86

Thus 0·01 mg. protected 86 % of the rats inoculated with it.

The solution was then allowed to autolyse for a month. It contained originally 2 mg. nucleoprotein per cubic centimetre. It was then filtered through 10 % gelatin under a pressure of 40 atmospheres. The particular method employed was that devised by Martin[1].

Under this pressure filtration was rapid and it was feared that the gelatin was not effecting a complete separation between the proteins

[1] *Journal of Physiology*, vol. xx. (1896) p. 364.

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and the solution. The solution was accordingly passed through the filter several times until a small portion of the filtrate gave no reaction on boiling after acidification with acetic acid. The apparent passage of the nucleoprotein through the gelatin might be used as an argument that the constituent molecular aggregates are small. It must be remembered that the solution was, considered as a proteid solution, very dilute. It contained originally only 2 mg. per c.c. and after the month's lysis it contained less. Thus the solution was only 0·2 %. The statements that are given as to the impermeability of 10% gelatin to protein solutions are all based on the examination of much stronger solutions than this. It is possible that very dilute solutions of all proteins pass through gelatin. Indeed, the fact that continued passage of the dilute solution used in this experiment did result in a more nearly complete arrest of the protein points to this conclusion.

After the filtration was complete a further test was made for the presence of protein. It was found that in 100 c.c. an amount of protein approximating to 1 mg. was present.

It now remained to determine the vaccinating value of this solution. The experiments performed to ascertain this point are given in the following table:

Dose	No. rats	No. survived	% survived
1 c.c.	19	16	84

The quantity of nucleoprotein contained in the vaccinating dose was 0·01 mg.

The amount of protection—84 %—is practically identical with the protection obtained by the use of the solution before hydrolysis.

Now the hydrolysis has increased the ratio of antigen to nucleoprotein, for it has diminished the amount of protein recoverable by boiling in the presence of acetic acid (*Journal of Hygiene*, vol. XI. (1911), supplement, p. 27), and it has not diminished the amount of antigen (*loc. cit.*). We should expect, therefore, that even if the gelatin has not diminished the ratio of protein to antigen, the filtrate would show a high immunising value. The experiment quoted does not determine the ratio of antigen to protein. The protection afforded is high—over 80 %. It was possible that a smaller quantity of the filtrate would give the same amount of protection. If a smaller amount of the filtrate was found to give a smaller protection then we must conclude that the ratio of antigen to nucleoprotein was not materially altered by the filtration. This, as the following experiments show, was found to be the case:

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Dose	No. rats	No. survived	% survived
1/20 c.c.	23	13	56
1/200 c.c.	23	13	56

Reference to the previous reports (vol. x. (1910), p. 559 and vol. xi. (1911), supplement, p. 40) will show the amount of protection following the inoculation of a one-month's hydrolysed extract. Thus on p. 40 of the second report 53 % survived after a vaccinating dose of 0·001 mg., an amount comparable with that present in the solution used above.

We must conclude that filtration through gelatin has effected no separation of antigen from protein.

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LVIII. BESREDKA'S METHOD OF VACCINATION.

By SYDNEY ROWLAND, M.A., M.R.C.S.

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A METHOD of vaccination that has achieved considerable reputation is that advocated by Besredka of the Pasteur Institute, Paris[1]. "Vaccination par les virus sensibilisés," as he terms his method, consists essentially in the use of killed cultures of the organism concerned. In this it does not differ, so far as concerns plague, from the method of Haffkine. But Besredka claims that, if the killed organisms are submitted to a preliminary soaking in a serum containing the specific antibodies to the organism concerned, these antibodies neutralise the toxic substances contained in the bacilli, which can then be used as a vaccine having the advantage of being atoxic.

The present position of the author of this method will be found described in the *Bulletin de l'Institut Pasteur* (1910), vol. VIII. p. 241, where he says "le vaccin antipesteux sensibilisé est depourvu de toute action toxique."

Now in the case of the plague bacillus we can extract the specific endotoxine (for rats), consequently we are in the position of being able to control this claim. Assuming that a given quantity of plague bacilli will normally yield a certain definite amount of endotoxine, then it is obvious that, if Besredka's explanation of the principles underlying his method be correct, the same quantity of plague bacilli will, after soaking in the antiserum, yield a smaller amount of endotoxine or none at all.

The following experiments were undertaken to determine

(1) The toxicity of a "whole" vaccine prepared according to Besredka's method.

(2) The amount of endotoxine that could be extracted from plague bacilli before and after soaking (sensitising) in antiserum.

[1] See the reviews by Besredka in *Bulletin de l'Institut Pasteur*, vol. VIII. (1910), p. 241 and vol. X. (1912), p. 529.

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Experiment I. Determination of the toxicity of a vaccine prepared according to Besredka's method.

In this experiment the relative toxicity of three vaccines is determined. The three vaccines are comparable in every way except that in the case of vaccine 1, the bacilli were soaked (sensitised) in an antiplague serum; in the case of vaccine 2 they were soaked in normal horse serum; and in the case of vaccine 3 they were soaked in physiological salt solution.

The serum employed in the case of vaccine 1 was prepared by immunising a horse by means of the endotoxine of the plague bacillus as already described (*Journal of Hygiene*, vol. XI. (1911), supplement, p. 11). At the time of the experiment this serum was of such a strength that one cubic centimetre neutralised 150 lethal doses of endotoxine. The proportion of serum to bacilli was so arranged that there was present in the quantity of serum employed sufficient antitoxin to neutralise four times the amount of endotoxine present in the bacilli.

These relations having been determined by preliminary experiments, 100 Roux bottles of agar were inoculated with plague and incubated for four days at 34° C. The bottles were then heated to 60° C. for an hour to kill the bacilli. This is the temperature and duration of heating employed by Besredka. The growth was washed off in salt solution (0·8 %) and the bacilli twice washed in salt solution in the centrifuge. After the last washing the bacilli were again centrifuged and 41 grammes of paste obtained. This paste contained 13·84 % of solids when dried at 105° C. Ten grammes of the paste were put in each of three flasks containing glass beads and there were added 35 c.c. of antiplague serum, normal serum and salt solution respectively. When a uniform distribution of the organisms throughout the respective fluids had been effected the flasks were put aside in a cool place till the following day. Meanwhile the quantity of bacilli in emulsion 3 was determined. It was found that the strength of this emulsion as determined directly corresponded with the strength as calculated from the analysis of the paste from which it was made. We may take it then that the paste of organisms was homogeneous, that the process of emulsification was adequate and that the three emulsions were comparable in the amount of organisms they contained.

The lethal dose of the three emulsions was then determined by subcutaneous inoculation into rats. Two rats were used for each dose :

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Dose mg.	Emulsion 1	Emulsion 2	Emulsion 3
10	Died 2 days	Died 2 days	Died 2 days
	,,	,,	Survived
9	,,	Survived	Died 2 days
	,,	,,	Survived
8	,,	,,	Died 2 days
	Survived	,,	,,
7	Died 3 days	,,	,,
	Survived	,,	Survived
6	,,	,,	Died 2 days
	,,	,,	,,
5	,,	,,	Survived
	,,	,,	,,
4	,,	,,	,,
	,,	,,	,,
3	,,	,,	,,
	,,	,,	,,
2	,,	,,	,,
	,,	,,	,,
1	,,	,,	,,
	,,	,,	,,

The lethal dose of the three emulsions does not differ to any great extent. Outside the error of this kind of experiment there is no difference discoverable between the lethal dose of the organism that had been soaked (sensitised) in the antiplague serum and those that had been treated simply with salt solution. A slight apparent difference is noticed in favour of the organisms that had been sensitised in the normal horse serum.

A repetition of this experiment showed no diminution of toxicity in the case of the bacilli treated with serum.

Experiment II. Estimation of the amount and toxicity of the endotoxine that can be extracted from plague bacilli before and after soaking in antiplague serum, normal horse serum and salt solution.

A paste of organisms was prepared as in the last experiment: 32·5 grammes of paste were obtained containing 15·6 % solids.

Three suspensions of the paste were made as in the last experiment.

Suspension 1 contained 10 gr. paste in 37·5 c.c. antitoxic serum.
,, 2 ,, ,, ,, normal horse serum.
,, 3 ,, ,, ,, salt solution.

The three suspensions were left in a cool place until the following morning. They were then centrifugalised and the paste of organisms washed free from serum proteins. The final washed paste obtained was mixed with twice its weight (in each case) of anhydrous sulphate of soda. The semi-fluid mass thus formed soon set to a solid mass which was reduced to powder. The full description of this process

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of extracting the endotoxine of the plague bacillus has already been given in these reports (vol. x. (1910), p. 553). The powder was dissolved in warm distilled water and in such a volume as to form a saturated solution of sodium sulphate. After filtering off the solution the residue on the filter consisting of plague bacilli, sensitised or not as the case might be, was extracted with distilled water. The same amount of water was of course used in each case. Three extracts were thus obtained which contained the endotoxine from the three portions of bacilli which had been treated in the three ways indicated. Each extract was filtered through hardened filter paper to remove the bodies of the bacilli and the three filtrates examined as follows.

First the amount of nucleoprotein in each was determined by boiling a portion after acidification with acetic acid, drying and weighing the precipitate obtained. The weights of nucleoprotein in the three extracts in milligrammes per c.c. was as follows:

No. 1	No. 2	No. 3
6·62	6·34	6·66

These figures are very similar. There is no appreciable difference in the weight of endotoxine (nucleoprotein) extracted in the three cases.

Having thus failed to find any difference in the quantitative yield of nucleoprotein in the three cases, it remained to ascertain whether there was any qualitative difference in the three extracts. For this purpose the lethal doses of the three extracts were determined on rats. For the purposes of this determination the three extracts were made up to such a strength that 5 c.c. of each extract contained 1 milligramme of nucleoprotein. The three solutions were inoculated subcutaneously into rats as follows, two rats being used for each dose:

Dose mg.	Extract 1	Extract 2	Extract 3
1·0	Died 1 day	Died 1 day	Died 1 day
	,,	,,	,,
0·8	,,	,,	,,
	,,	,,	,,
0·6	,,	,,	,,
	,,	,,	,,
0·4	Died 2 days	,,	,,
	Survived	Died 2 days	,,
0·2	Died 1 day	Died 1 day	,,
	Died 2 days	Survived	Died 3 days
0·1	Survived	Died 1 day	,,
	,,	Survived	Survived

The minimal lethal dose of the nucleoprotein obtained from bacilli that had been sensitised in immune horse serum was thus substantially

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the same as the minimal lethal dose of the nucleoprotein obtained from the bacilli that had been treated (sensitised) in normal horse serum or in saline. The yield of nucleoprotein was also the same in the three cases.

From this experiment, which was repeated with similar results, it is concluded that on sensitising the plague bacillus in an antitoxic serum no permanent neutralisation of the endotoxine takes place.

The control experiment in which the bacilli were treated in salt solution shows that the effect of sensitising in either anti or normal serum is nil as regards the amount of nucleoprotein or the toxicity of the nucleoprotein.

I am therefore unable to confirm Besredka's statement that sensitised organisms yield an atoxic vaccine, for the whole organisms after sensitisation are just as toxic as before and no neutralisation of the endotoxine takes place as the result of the sensitising.

As the immune serum neutralised the endotoxin after extraction from the bacilli it must be concluded from these experiments that the antitoxine cannot enter the bodies of bacilli killed by heat.

There is, however, evidence that Besredka's vaccine is possessed of good immunising power and users of it have reported favourably as to the minimum of discomfort following its inoculation. A great point of this latter property is made by Besredka. Paladino-Blandini (*Annali d'Igiene sperimentale* (1905), pp. 295, 411), after an exhaustive examination of several methods of antityphoid vaccination, speaks in terms of high praise as to this property of Besredka's vaccine. Dopter (*Annales de l'Institut Pasteur* (1909), vol. XXIII. p. 677; *C. R. Soc. Biol.* vol. LXIV. (1907), p. 379), working with dysentery, speaks to the same purpose.

Besredka attributes the advantages of his sensitised vaccines firstly to an actual neutralisation of the endotoxin and secondly to sensitisation (opsonisation) of the bacteria. For the former I can find no support from my experiments with plague, but immune horse serum does contain opsonin. At one time Besredka recommended the use of normal horse serum in the preparation of his sensitised vaccine as being equally efficacious. Normal serum, however, can hardly be supposed to be antiendotoxic or possessed of any considerable specific opsonising power.

A further possibility is that after soaking in immune serum the bacilli are more readily lysed when placed under the skin. In this respect treatment with normal horse serum may be advantageous, for

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whilst engaged in some investigations into the mechanism of plague immunity I found that in the normal horse a natural amboceptor for the plague bacillus exists in considerable quantity. It is possible that in Besredka's method this natural amboceptor is responsible for sensitising the bacilli so that they dissolve more rapidly after injection. Whilst at first Besredka employed specific sera for his sensitisation, later he abandoned these for the use of normal horse serum. Later still he reverted to the use of specific sera and makes the statement that it is necessary for these to be highly agglutinating.

The main experiments that led to the recognition of the normal amboceptor in the serum of normal horses are as follows:

(1) 80,000,000 virulent living bacilli were added to 1 c.c. of fresh normal horse serum. The serum was kept at 37° C. No multiplication took place and at the end of 24 hours the serum was sterile.

(2) 83,000,000 living virulent bacilli were added to 1 c.c. of the same serum as was used in the last experiment. The serum had been heated to 58° C. for one hour. Multiplication took place and followed the same curve when the numbers were plotted against time as the same number of bacilli inoculated into broth.

(3) 10,000,000 living virulent bacilli were added to one c.c. of the same serum that had been heated. At the same time 0·04 c.c. of fresh normal rat serum was added to the tube. No multiplication took place and at the end of four hours only half a million bacilli were recognised.

The growth and fate of living plague bacilli were traced in horse serum by the use of a method of direct microscopical observation. Specially constructed observation slides were used which, combined with a very perfect method of dark ground illumination, allowed of a record of the appearances of the living organisms and of their number being made as time advanced (see below, p. 362).

The further fact that so far as they have been examined all antiplague sera prepared by Yersin's or some equivalent method are strikingly deficient in specific plague antitoxine supports the explanation above given.

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LIX. THE RELATION OF PSEUDO-TUBERCLE TO PLAGUE AS EVIDENCED BY VACCINATION EXPERIMENTS.

By SYDNEY ROWLAND, M.A., M.R.C.S.

Of the Lister Institute.

The resemblance of the bacillus of pseudo-tubercle of rodents to that of plague is very striking. The work of Galli Valerio[1], Zlatogoroff[2], and MacConkey[3] bears evidence of this. It has even been suggested that the pseudo-tubercle bacillus should be rechristened and be known as the pseudo-plague bacillus. There is no constant morphological or staining character which will enable us to distinguish the one from the other, nor do we receive much assistance towards the attainment of this end from a study of the cultural and biological properties of the two organisms[4]. The differences are merely differences of degree. It is the same with regard to the agglutination, precipitin and, as far as they are known, complement deviation reactions (J. Henderson Smith[5]). The post-mortem appearances in both diseases may be so strikingly similar that one can do no more than hazard a guess as to which bacillus is the cause of them. The similarity in the appearances of a guinea-pig that has died of pseudo-tubercle to those of a guinea-pig that has died of plague is so great as often to amount to identity. The only well-defined difference between the two organisms is one of virulence, for whereas the rat is hardly, if at all, susceptible to pseudo-tubercle it is very susceptible to plague. The guinea-pig is usually regarded as being equally susceptible to either.

These great resemblances led Zlatogoroff and MacConkey to test the question of the possibility of immunising rats and guinea-pigs against plague by means of the pseudo-tubercle bacillus. Zlatogoroff failed to obtain cross-immunisation but MacConkey concluded that it

[1] *Cent. f. Bakt. Orig.* vol. xxxiii. (1903), p. 321. [2] *Ibid.* vol. xxxvii. (1904), p. 513.

[3] *Journal of Hygiene*, vol. viii. (1908), p. 335.

[4] MacConkey, *Journal of Hygiene*, vol. v. (1905), p. 350; see also *ibid.* vol. viii. (1908), p. 305.

[5] Unpublished observations.

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was possible to immunise both rats and guinea-pigs against plague by means of inoculation of cultures of the pseudo-tubercle bacillus. In the case of rats he employed a living culture as his vaccine, whilst he found that with guinea-pigs either a living or a filtered autolysed extract was efficaceous. His conclusions being drawn from only a relatively small number of animals it was considered of importance that they should be confirmed or refuted, and if found to be correct that the scope of the enquiry should be extended so as to obtain amongst other things some estimate of the extent of the immunity produced.

The immunity to plague conferred on guinea-pigs by pseudo-tubercle.

A vaccine was prepared from a culture of pseudo-tubercle that came originally from Professor Pfeiffer.

Preparation of the vaccine. Twelve Roux flasks were inoculated with this culture and incubated for three days at 35° C. Five c.c. of chloroform were then introduced into each flask and the flasks were again incubated for four hours. At the end of this time 10 c.c. of salt solution were introduced into each flask and the growth emulsified. To 26 c.c. of this emulsion were added 134 c.c. of salt solution. This constituted the vaccine. Each guinea-pig received 0·5 c.c. of this vaccine subcutaneously and after an interval of 15 days a second dose of 1·0 c.c. It was reckoned that the first dose corresponded to $\frac{1}{8}$th agar tube and that the second dose corresponded to $\frac{1}{4}$th agar tube of average growth.

A month after the second vaccination each pig received $\frac{1}{10}$ c.c. of a broth culture of living virulent plague subcutaneously. At the same time 10 control pigs received a similar dose.

There were 44 guinea-pigs vaccinated and not a single one died of plague or was noticed to be even ill, whereas nine out of the ten control pigs died of plague. The vaccination has, therefore, given rise to complete protection.

The immunity to plague conferred on rats by pseudo-tubercle.

MacConkey obtained indications of some degree of resistance to plague in rats which had previously been inoculated with living pseudo-tubercle bacilli. Of thirteen rats which were so treated five died of plague when tested five months after the last immunising injection. Of his five controls all died.

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Accordingly an experiment was made on rats using the same vaccine and the same method of immunisation as in the case of guinea-pigs. Thirty-seven rats were used for the experiment, but no evidence was obtained that any protective effect was produced when tested with living virulent plague four weeks after the second dose of vaccine.

The difference in the reaction of the two classes of animals to the vaccination appears all the more remarkable when we remember the facility with which rats can be immunised against plague by previous treatment with dead plague cultures and the difficulty with which by this means guinea-pigs are rendered immune to the same disease.

The immunity to plague conferred by the nucleoprotein of the pseudo-tubercle bacillus on rats and guinea-pigs.

As in the case of the plague bacillus a water-soluble nucleoprotein can be extracted from the bacillus of pseudo-tubercle. The same methods which have already been described in these reports (this *Journal*, vol. x. (1910), p. 553) were applied to the pseudo-tubercle bacillus and an extract prepared similar in its chemical characteristics to that obtained from the plague bacillus. Thus, to give an example of one preparation, 19 Roux bottles were inoculated and after four days' incubation at 34° C. a good growth was obtained. This was swept off in salt solution and the bacilli centrifuged down. The paste of organisms was emulsified in salt solution and again centrifuged in order to wash the bacilli. This process was repeated again. No chloroform was used in the process. To the final washed paste an equal weight of anhydrous sulphate of soda was added. The semi-liquid mass thus produced was rubbed in a mortar until on cooling it became a dry powder. The powder was dissolved in twice its weight of warm distilled water. A solution of sulphate of soda is obtained by this means which is of such a strength as to completely precipitate all the bacterial proteins. This solution was filtered off and the residue of organisms suspended in distilled water to extract. After an hour the extract was filtered through hardened paper and a clear fluid obtained. This clear fluid was found to contain 10·8 mg. of nucleoprotein per c.c. Unlike the extract obtained from the plague bacillus by the same treatment it was without toxicity for either rats or guinea-pigs. Guinea-pigs and rats survived doses of 10 mg.

Numerous experiments were made to determine whether this extract was possessed of any immunising value against living plague but all with a negative result. Thus, in one series of 26 rats the mortality

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of the vaccinated animals was 18 % greater than the mortality of the controls and in another series of 26 guinea-pigs it was only 6 % less. In both these series the vaccinating dose of the nucleoprotein was 0·01 mg. and the test dose of plague was given two weeks after the vaccination.

Further experiments were made under the same conditions of testing as have been used in determining the immunity against plague following the inoculation of various vaccines, the technique of which is detailed in the first of these reports. The results may be tabulated as follows:

	Dose	No. animals	Result	% mortality
Rats	0·1 mg.	23	12 died of plague	52
	0·01 ,,	5	5 ,, ,,	100
Guinea-pigs	0·1 ,,	22	13 ,, ,,	59
	0·01 ,,	28	19 ,, ,,	68
Controls not vaccinated:				
Rats		19	14 ,, ,,	73
Guinea-pigs		15	9 ,, ,,	60

In the case of rats which received the 0·1 mg. dose a slight immunity is noticed.

In the case of the guinea-pigs no immunity is evident.

We must conclude that by the method employed the substance contained in the pseudo-tubercle bacillus which immunises guinea-pigs against plague cannot be extracted.

If it cannot be extracted and if it is not destroyed by the process used in attempting to extract it, it must be insoluble in dilute saline and remain in the bodies of the bacilli after the extraction. No experimental determination of this question has yet been made, but assuming that it remains in the bodies of the bacilli it presents a striking contrast to the antigen of plague which is effective in the case of rats.

The plague antigen for rats is easily extracted from the plague bacillus, which bacillus under similar treatment yields little or no antigen for guinea-pigs. A plague antigen for guinea-pigs is contained in the pseudo-tubercle bacillus from which by the method employed it cannot be extracted. The antigens for the two animals are, therefore, different.

The mechanism of immunity in the two classes of animals is therefore different—a fact of great importance in the art of vaccination. And it therefore behoves us to be careful in applying the results obtained from the study of a particular species of animal to man.

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Note on the toxicity of the plague nucleoprotein for guinea-pigs. The nucleoprotein obtained from the plague bacillus and which, as has been shown, is invariably toxic and possessed of antigenic powers for rats is almost without action on guinea-pigs.

This conclusion is the result of repeated trials. The following test of a sample that was prepared without chloroform in order to eliminate the possible neutralising action of this substance may be given:

Dose	Rats	Result	Dose	Guinea-pigs	Result
5·4 mg.	1	Died 3 hours	27 mg.	1	Survived
	2	Died 1 day		2	,,
2·7 ,,	3	,,	18 ,,	3	,,
	4	,,		4	,,
1·3 ,,	5	,,	2·7 ,,	5	,,
	6	,,		6	,,
0·1 ,,	7	,,	1·3 ,,	7	,,
	8	,,		8	,,

Coincident with this absence of toxicity, there is a minimum amount of protective power as the following table shows:

No. guinea-pigs	Protecting dose	Result	% survived
20	11 mg.	14 died	30
20	5 ,,	17 died	15
Controls received living plague only:			
40	—	37 died	7

The slight balance in favour of the vaccinated animals is not significant in view of the fact that pseudo-tubercle is a common disease amongst laboratory guinea-pigs and the knowledge we now have as to the protective value of this bacillus against plague.

Summary.

It will be as well to summarise the above facts:

1. *The plague bacillus is equally virulent for guinea-pigs and rats, but whereas it is easy by either the whole bacillus or the contained nucleoprotein soluble in saline to immunise the rat against plague it is almost impossible to immunise the guinea-pig, either by means of the bacillus or its nucleoprotein.*

2. *The pseudo-tubercle bacillus is almost without pathogenicity for rats whereas the guinea-pig is susceptible to it.*

3. *It is easy to immunise the guinea-pig against plague by means of the pseudo-tubercle bacillus; very difficult to immunise the rat.*

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4. *It was not found possible to immunise either the rat or the guinea-pig against plague by means of the nucleoprotein contained in the pseudo-tubercle bacillus.*

5. *Whereas the nucleoprotein of the plague bacillus grown on broth agar is toxic for rats it is hardly so at all for guinea-pigs. The nucleoprotein obtained from the pseudo-tubercle bacillus is relatively innocuous to either rats or guinea-pigs.*

These facts suggest that for a bacillus or bacillary product to act as an efficient vaccine it must in its original condition be possessed of toxic action for the animal to be protected. Thus the plague bacillus is toxic for rats and can be used as a vaccine, the nucleoprotein obtained from it is toxic for rats and will protect them, but it is not toxic for guinea-pigs and will not protect them. The pseudo-tubercle bacillus is pathogenic for guinea-pigs and will protect them, the nucleoprotein obtained from it is toxic to neither rats nor guinea-pigs and is incapable of protecting either.

This statement appears at first sight as opposed to the facts established in a previous report as to the independence of the toxic and antigenic properties of the nucleoprotein obtained from the plague bacillus, but this is not so. The mother substance from which the efficient vaccine is to be obtained must be of a toxic nature towards the animal it is desired to protect, but it is possible by hydrolysis to abate or destroy its toxicity without interfering with its antigenic properties. It would seem as if the antigenic and toxic properties of the nucleoprotein were closely connected—possibly a common molecular grouping is an essential part of both.

In this connection we are reminded of the established relations that exist in the case of diphtheria between toxins and toxoids. Diphtheria toxin on keeping spontaneously degenerates into toxoids. The endotoxine of the plague bacillus does the same, that is if we admit that the atoxic product of the hydrolysis of the toxin is analogous to the toxoid of diphtheria. These analogies are sufficiently apparent, but it should be noted that whereas in the case of plague the toxoid—so to name it—produces in animals not only antitoxines but antibacterial bodies as well, in the case of diphtheria the toxoid, and for that matter the toxin itself, produces only antitoxic substances.

An apparent conversion of a virulent plague bacillus into the bacillus of pseudo-tubercle.

These experiments and the conclusions based thereupon emphasise the close connection existing between the bacillus of plague and that

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of pseudo-tubercle. The only test capable of distinguishing between the two organisms is an inoculation test. Pseudo-tubercle will not kill rats with the signs and symptoms of plague. To this may perhaps now be added that the nucleoprotein obtained from the pseudo-tubercle bacillus will not immunise rats against plague. But if the animal test fails us, how are we to distinguish between the two organisms? In that case it would appear that there is no means of settling the question. If two organisms from different sources cannot be distinguished by any known test it must for the time being be assumed that the two organisms are identical. If then we can so manipulate the bacillus of plague as to make it conform in its behaviour towards animals in all respects with that of the bacillus of pseudo-tubercle, then we must conclude that the two organisms are fundamentally the same, and relegate the difference in their effect on animals to the category of virulence.

We now proceed to show that by appropriate treatment the differences between a plague organism and the bacillus of pseudo-tubercle as regards their effect on animals (rats and guinea-pigs) can be made to disappear.

Some years' experience in the maintenance of a virulent strain of plague at its full virulence over a long period of subculture has suggested the following procedure. The culture is kept under such conditions as reduce the rate of growth to a minimum, and when it is required for test purposes it is put under such conditions as make the rate of growth a maximum. This amounts to keeping the stock culture cool and immediately before use transferring it to fresh medium at the optimum growing temperature. Any conditions which allow the bacilli to grow in the presence of their own products seem to lower the virulence and *vice versa*.

In conformity with this experience the experiment was tried of growing the stock virulent plague culture in a solution of the nucleoprotein obtained from the plague bacillus itself. Good growth took place. After two days' growth the culture was inoculated into a series of rats and guinea-pigs with the following results:

Ten animals were used in each series. Among the ten rats no acute deaths took place. One died on the fifth day with no signs of plague, three others died on the second, eighth and tenth days respectively. In none of them was there any signs of plague. Of ten control rats inoculated at the same time with the same culture grown in ordinary broth seven died of acute typical plague.

Growing the virulent bacillus in its own nucleoprotein has thus

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abolished the virulence of the organism as regards rats. The dose of the cultures (0·1 c.c.) administered was the same in both cases[1].

It would seem then that the effect on rats of the inoculation of a culture grown in a solution of its own nucleoprotein is the same as would be produced by the inoculation of a typical culture of pseudo-tubercle, the usual results of such an inoculation being the death of a certain number of the rats without any noteworthy signs. From such inoculated rats it is impossible to recover the pseudo-tubercle bacillus[2].

Now as to the effect on guinea-pigs of the same culture.

Ten guinea-pigs were inoculated with the same dose as the ten rats. Of these ten pigs all died. The days on which they died were the eleventh (three), fifteenth and seventeenth respectively. On the fifteenth day five pigs were so ill that they were killed. All were examined post-mortem. In all the appearances were the same, viz. those typical of pseudo-tubercle. In this opinion Dr MacConkey, who examined the animals, quite agreed.

Ten control guinea-pigs inoculated with the same culture grown in ordinary broth gave the following results: nine died, the times of death being 6, 8, 6, 7, 6, 3, 5, 5, 5 days respectively.

By growing the virulent bacillus in the solution described it appears that not only is the day of death postponed, which is characteristic of pseudo-tubercle, but in addition the post-mortem appearances have changed from those typical of acute plague to those typical of pseudo-tubercle. This latter point must not be pressed too far as it is often impossible to distinguish between the two appearances. A known culture of plague will sometimes kill guinea-pigs with post-mortem signs indistinguishable from those of pseudo-tubercle.

The broad fact remains that in these experiments we have converted the bacillus of plague which kills rats acutely into a bacillus which will hardly kill them at all and then without any of the post-mortem signs of plague. On the other hand as regards guinea-pigs we have converted the acute killing bacillus into a chronic killing bacillus. In fact the bacillus that is found in the culture of the plague bacillus in the nucleoprotein solution is indistinguishable from a typical pseudo-tubercle bacillus.

[1] Even if the density of the cultures was not so nearly the same as it appeared to be, it has already been shown (vol. x. p. 543) that the fatality does not vary much with the dose within wide limits about $\frac{1}{10}$ c.c.

[2] Personal communication by Dr MacConkey, and Dr Martin tells me he has had the same experience.

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LX. OBSERVATIONS ON THE MECHANISM OF PLAGUE IMMUNITY.

By SYDNEY ROWLAND, M.A., M.R.C.S.

Of the Lister Institute.

The fate of the plague bacillus after inoculation into rats.

I. Peritoneal inoculation.

1. *The mechanism in normal animals.*

The introduction of plague bacilli into the peritoneal cavity is followed by a reaction which is typically inflammatory. The first effect is a secretion of fluid containing cellular elements. The fluid is rich in a fibrin-forming mechanism, and the cells are so numerous as to give the fluid a milky appearance. They are during the first hours principally mononuclears and lymphocytes, the polynuclears appearing in force about the third hour. The cells are actively phagocytic but not uniformly so. It is common to find amongst a mass of polynuclears a minority that are crowded to bursting point with bacilli and a majority that are quite free from bacilli. Examined microscopically (on the warm stage) both are found to be actively amoeboid so that there is no question of killing by, for example, endotoxine. The fact is difficult of explanation.

The fluid, as has been remarked, readily clots. This clotting power forms the basis of a mechanism of great beauty. If a little of the exudate be removed and examined microscopically on the hot stage, delicate fibrils of fibrin are seen to form and to traverse the field in all directions. To these fibrils the bacilli become attached. So marked is this effect that the first evidence of the formation of the fibrils is often the arrangement of the bacilli in lines. A network of fibrils is thus formed traversing the fluid and enclosing in its meshes bacilli and cells. Soon the network, or more aptly the spongework, as it is extensive in all dimensions, begins to contract. The contraction continues until there results a mass of cells, bacilli and fibrin inextricably entangled.

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In this way are formed in the peritoneal cavity small flecks floating in the exudate. Many of the cells are full to bursting point with organisms, others are plasmolysed setting free their granules, a few appear healthy. In some cases it appears as if so many cells have coalesced that the bacilli are lying in a syncytium of cells. Prominent in such a mass of cells are the eosinophile granules and the large basophil granules characteristic of the rat. The whole process is, in its first effects, a very perfect netting mechanism, resulting in a close aggregation of cells and bacilli. The effect is to clear the peritoneal cavity of bacilli and to bring them into intimate association with inflammatory cells. In this process the omentum plays a part. The ultimate fate of the flecks as I have called them above is to become attached to the omentum. If sufficiently numerous the omentum becomes retracted and rolled up enclosing masses of the flecks. In time, if the animal survives, the centre of the mass may become an abscess cavity.

In the course of the above process the animal may die at any stage. This death is presumably due to the absorption of endotoxine, for invasion of the body by bacilli has not yet taken place. At any time during the process there are observed bacilli which are free and have escaped the netting, and the fate of the animal depends on the completeness with which the process can accomplish itself. On the one hand we have the process as described progressing in an orderly sequence, on the other we have the bacilli multiplying at a certain rate. The fate of the animal depends on these two rates. If the rate of the process can compete successfully with the rate of multiplication then the animal survives, if on the other hand the rate of multiplication of the bacilli is greater than the mechanisms of netting, lysis and phagocytosis can compete with, then the animal succumbs to plague.

2. *The mechanism in immune animals.*

In an immune animal (*i.e.* an animal that has been vaccinated so that out of 100 animals so vaccinated 80 survive a certain lethal dose of virulent plague) precisely the same course of events takes place. At any stage the differences observed are not differences of kind but of degree. Thus whereas in the normal animal the number of bacilli escaping the process are many, in an immune animal there will be no bacilli outside the net. The extent of the reaction in the normal and immune animals seems to be the same and to depend more on the quantity of bacilli present than on anything else. *The number of bacilli on the other hand in the case of the immune animal is at any stage of the*

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process much less than is the number at the same stage in the case of the normal, unvaccinated animal.

The differences observed in the two cases are all explicable on the assumption that in the case of the immune animal the multiplication of the bacilli is much less than in the case of the normal animal.

II. Subcutaneous inoculation.

As peritoneal infection is hardly a natural mode of infection, it was possible that the conclusions drawn from its study might be erroneous. The usual method of infection in plague is a cutaneous or a subcutaneous inoculation, both in the laboratory or in the natural epidemic. A series of rats were accordingly inoculated subcutaneously with virulent and avirulent plague. A method had to be devised for following the course of events. It was possible to cut series of sections and to study them at leisure. A consideration of the difficulty and labour of getting a comprehensive view of the course of events from some thousands of sections led to its abandonment and the following method was employed. A rat was inoculated subcutaneously, taking care that the dose was deposited close under the skin, so that it might be possible to recover the bacilli and exuded cells from the point of inoculation, and to examine the condition of affairs by the method of smears. This method has the advantage that a living preparation may be put up and examined on the hot stage at any time. It is essential that the bacilli should be deposited in a limited area which can be subsequently identified. This is best ensured by inserting the needle from the opposite side of a limb until its point can be felt pushing up the skin on the other side. About $\frac{1}{10}$ c.c. of culture is then deposited close under the skin at the point selected. The needle is then withdrawn through the muscle of the limb.

When it is desired to examine the course of events the whole skin is reflected including the subcutaneous tissue. The point at which the bacilli were deposited is then recognised through the fat and areolar tissue as a whitish spot. This is opened and smear preparations made and stained, usually with Leishman's stain. Apart from the usual and well known inflammatory reaction which is common to the presence of any foreign body the following specially interesting points were observed.

The difference between the reaction in the case of the normal and the immune rat was, as in the case of the peritoneum, one of degree and not of kind. Whereas in both cases the bacilli increased, in the

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case of the immune rat the number of bacilli recoverable at any stage of the process was always much less. Concurrently the extent of the reaction in the immune rat is always much less than in the case of the normal rat, the extent of the reaction depending, other things being equal, on the number of bacilli present.

The conclusion reached was that the rate of increase of the bacilli in the normal rat was always much greater than was the rate of increase in the case of the immune rat. This conclusion may be expressed in another way by saying that the normal rat provides a good culture medium for the plague bacillus whereas the immune rat provides a very bad one.

One series of rats examined in this way may be especially alluded to. It was a series inoculated with a strain of plague that had become, on long subculture in the laboratory, almost completely avirulent. The reaction in this case was of the same typical inflammatory character as in the case of the virulent bacillus. Day by day it was possible to examine the condition of affairs for a week. This was possible as the bacillus being avirulent the rats did not die. For as long as four days after the inoculation free bacilli were found at the site of inoculation. A certain amount of phagocytosis had taken place but this was not complete, bacilli were still after this long time slowly multiplying, unattacked by the phagocytic process. Under the same circumstances of inoculation but with a virulent bacillus the rat would in all probability have been dead by the end of the fourth day. There would have been found at the site of inoculation a vast number of bacilli with a correspondingly extensive reaction. This reaction would not have differed in kind from the small reaction observed in the case of the avirulent bacillus. The difference in the two cases impressed on one is that the virulent bacillus can multiply readily in the body of the rat whereas the avirulent organism can only do so exceedingly slowly or not at all.

In the same way a virulent organism multiplies slowly in the body of a vaccinated rat and rapidly in the body of a normal rat. As regards the virulent organism in the body of the rat the effect of vaccination has been to convert the picture into that of an avirulent one in a normal rat.

It is impossible to give a quantitative measure of these experiments but from the examination of a large number of animals the conclusion is forced upon me that *the essential factor in plague immunity is one which affects the multiplication of the bacillus.*

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The multiplication of bacilli in rat serum.

We must now examine this question specifically and determine whether there is a difference in the rates of multiplication of plague bacilli in the body fluids of normal and immune rats. It will be convenient to begin with the serum.

Method of investigation. A method is required that will give at any moment the bacilli existing in the serum under examination. Plating was out of the question as it was soon found that the bacilli grew in chains when the medium contained serum, and one could not by plating discriminate between one bacillus and several hundred bacilli under these circumstances. The same applies to a dilution and end point method. The only method available is direct counting under the microscope. This involves the examination of a given volume spread in a layer of uniform thickness and an illumination capable of defining the individual elements distinctly. The first method tried was to use a Thoma counting cell as in the enumeration of blood elements and dark ground illumination with a paraboloid condenser. A difficulty arose owing to the fact that in serum the organisms settled very slowly; under these circumstances it was necessary to count throughout the depth of the cell and when the focus was adjusted to any one point save the bottom, on which the cross lines are engraved, the diffraction rings from these lines obscured the images of the bacilli. In addition the obliquity of the illuminating cone in the case of the paraboloid is so excessive that if the medium was not optically clear the diffracted images of the suspended particles further obscured the images of the bacilli. These difficulties were overcome by having special counting chambers made (by Zeiss) without any rulings. The place of the rulings was taken by two spider lines in the eyepiece. These are adjustable and can be set at any desired interval. They always remain in focus and the entire depth of the cell can be examined and the bacilli at all levels counted.

The difficulty of the obliquity of the illumination was overcome by discarding the paraboloid and using in its place the aplanatic condenser of Zeiss to which was adapted a suitable central stop. Most of the counts were made with the 8 mm. apochromatic objective of Zeiss with a central aperture stop. Some of the counts were made with the 4 mm. of the same series.

The dark ground effect with this arrangement is very perfect. The

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bacilli stand out as bright rings on a dark ground and no difficulty arises from impurities in the medium, at least these difficulties are minimised. The depth of the cell is constant and known, the width is read off from the screw adjusting the distance apart of the spider lines in the eyepiece, the length is measured by a vernier attached to the mechanical stage. Thus the volume counted over is accurately known, and the number of organisms per unit volume can be expressed in any convenient terms. In what follows the units are millions per cubic centimetre.

If a small number of plague bacilli that have been grown on agar be introduced into fresh normal rat serum, growth as a rule fails to occur. If the fate of the introduced bacilli be watched hour by hour they are seen to gradually lose their bright outline (bright under the conditions of illumination employed) and to finally become indistinguishable. At the same time they lose their power of being stained. If a larger number be introduced the same fate befalls a proportion of them, the remainder after the lapse of some hours beginning to grow in chains. The proportion that begin to grow after surviving the lytic power of the serum is surprisingly small. Thus of 20 million organisms that were placed in fresh normal rat serum, only half a million were found after 10 hours' incubation at 37° C. The recognition of the living organisms under these circumstances is facilitated by counting the number of multiplying units. Thus in the case mentioned 20 million individual organisms were introduced, the bacilli being obtained from the surface of agar and emulsified in salt solution by the aid of shaking with beads until no clumps remained. After 10 hours, multiplication of the survivors in the serum had commenced and small chains of three or four organisms were seen. A count of the number of these chains gives the number of the individuals that survived the lysis in the normal serum. This lytic effect of normal serum is an obstacle in investigating the growth of plague in immune serum.

As we have seen, the plague bacillus contains within its body an endotoxine. On the occurrence of lysis this endotoxine is liberated and if an immune serum is under investigation the liberated endotoxine will neutralise the antibodies present in the serum. Thus the very substances whose presence it is desired to investigate become wholly or partially neutralised at the outset and the maintenance of a clean experiment becomes impossible.

This lytic effect of normal serum is not confined to plague; in

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typhoid, for example, it constitutes a grave source of inaccuracy in the opsonic technique. The difficulty in this case has been overcome by the introduction of a dilution method. The adoption of such a method in the investigations with plague was not considered advisable as it was desired to investigate the growth of the organism under conditions as closely approximating to those obtaining in the body as possible. Another way out of the difficulty suggested itself. From one point of view the effects of the preliminary lysis may be regarded as breeding out from the agar or broth strain of organisms a serum-resistant race. This point of view is rendered probable from the consideration of some facts as to the numbers of bacilli lysed according to the number introduced into the serum. If the lytic process were one concerned with specific antibodies these would be used up in the process and the introduction of a sufficiently large number of organisms would exhaust them. This is not found to be the case, the number lysed being approximately a function of the number introduced. There is thus a selection among the bacilli, some of them being capable of growing in serum and others not.

On the other hand this lysis in normal serum appears to depend on the presence of fresh complement as it can be abolished by a preliminary heating of the serum. Eight million organisms were introduced into one cubic centimetre of rat serum that had been heated to 55° C. for half an hour, multiplication commenced and at the end of four hours the number had increased to 30 millions. In a parallel experiment with the addition of 0·02 c.c. of fresh rat serum there was no multiplication at the end of four hours.

A method of circumventing the complicating process of lysis in fresh normal serum is as follows. If a certain number of bacilli be introduced into fresh normal serum the survivors begin to multiply in chains which can be recognised at the end of say 10 hours as being of a certain length or composed of a certain number of organisms. The rate of multiplication of the survivors, as indicated by the length of the chains after the expiry of a certain time, is accelerated by the presence of endotoxine. Thus, in a certain experiment 20 million organisms were added to one cubic centimetre of fresh normal rat serum, and at the end of 10 hours the number was reduced to half a million and the survivors had not begun to multiply. In parallel experiments in which varying amounts of endotoxine had been added to a series of tubes containing the same amount of serum and bacilli the results were as follows:

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Amount of endotoxine	Appearance of survivors
0 mg.	No chains
0·02 ,,	No chains
0·04 ,,	No chains
0·08 ,,	Chains of four or six
0·16 ,,	Very long chains

A preliminary lysis occurs, after which the rate of growth of the survivors is accelerated by the presence of the endotoxine. The fact that the endotoxine can be added in increasing amount before this effect is noticeable, suggests that a certain proportion is combined with the serum before its accelerating effect becomes apparent. If this experiment be repeated with immune serum the same effect is observed with this important distinction that the amount of endotoxine that must be added to produce the accelerating effect is very much larger. Thus in one experiment with fresh immune rat serum to which 10 million organisms had been added at the expiry of 10 hours the following effects were observed:

Amount of endotoxine	Appearance of survivors
0 mg.	No organisms recognisable.
0·2 ,,	,, ,, ,,
0·4 ,,	0·06 mill. multiplying elements in short chains.
0·8 ,,	0·1 mill. elements in short chains.
1·6 ,,	0·2 mill. elements in long chains.
3·2 ,,	0·7 mill. elements in very long chains.
6·4 ,,	Luxuriant growth in chains and masses too numerous to count.

The appearance in the tube that had received 0·8 mg. in the case of the immune serum was similar to that in the tube that had received 0·08 in the case of the normal serum. These experiments were repeated with similar results. It would appear that the endotoxine of the plague bacillus prepares the serum in some way, rendering it a good culture medium for the organism and that this effect is neutralised by immune serum.

These experiments are incomplete. So far as they have been carried however they give some indication of the mechanism of the immunity conferred by the inoculation of the endotoxine.

The characters of this immunity as evidenced in the suggestive experiment last mentioned give ground for regarding it as an anti-endotoxic immunity. On this view the function of the endotoxine of the plague bacillus is to counteract the disadvantageous effect of some constituent of the serum and to render the rat a good culture medium for the bacillus. How it accomplishes this is not clear; it may well

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be that the proteolytic enzyme contained in the bacillus and always present in the endotoxine plays a part. If so it must have a very high velocity coefficient. In experiments that have been made to test the anti-endotoxic power of horse serum it apparently makes little difference whether the mixture of toxin and serum is allowed to stand for more or less time before inoculation into the test animal.

Whatever the mechanism, the fact remains that the same toxine that is capable of provoking the formation of antitoxine in the horse is capable of neutralising it in the immune serum of this animal, while when it is injected into rats it provokes an immunity to infection with the living organism, the essential features of which seem to be resident in the serum of this animal, and to be in turn capable of being neutralised by the endotoxine.

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LXI. THE ONSET AND DURATION OF THE IMMUNITY CONSEQUENT ON THE INOCULATION OF PLAGUE NUCLEOPROTEIN.

By SYDNEY ROWLAND, M.A., M.R.C.S.

Of the Lister Institute.

It has been shown in a previous report (vol. x. p. 559) that consequent on the inoculation of the nucleoprotein obtained from the plague bacillus an immunity is established to the subsequent inoculation of living plague bacilli in broth culture. Some indication of the extent of this immunity was obtained by comparing the mortality of the inoculated rats after the test inoculation with the mortality that occurred (78 %) in uninoculated rats. In all these investigations the time that was allowed to elapse between the protective inoculation and the test inoculation was 14 days. We have now to consider the influence of the length of time elapsing between the protective and test inoculations upon the protection.

It has also been shown that the extent of the protection consequent on the inoculation of the nucleoprotein is of the same order in the cases where this substance is employed as a vaccine either in its fresh toxic condition or in its autolysed and relatively atoxic condition (vol. xi. supplement, p. 38). These two conditions of the nucleoprotein will also be considered in relation to the onset of immunity.

Onset of the immunity.

I. *Immunity conferred on rats by inoculation of the nucleoprotein immediately after, before or simultaneously with the test inoculation.*

A. Using fresh toxic nucleoprotein in 0·01 mg. doses:

of 10 rats inoculated 4 hours before the test dose 7 died 3 survived
" " " at the same time as the test dose 6 died 4 survived
" " " 5 hours after the test dose 7 died 3 survived
" " " 18 " " " " "

B. Using 2 months autolysed nucleoprotein in 0·01 mg. doses:—

of 10 rats inoculated 4 hours before the test dose 6 died 4 survived
" " " at the same time as the test dose 8 died 2 survived
" " " 5 hours after the test dose 8 died 2 survived
" " " 18 " " " " "

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From these experiments the conclusion is justified that the inoculation of the nucleoprotein either in its toxic or atoxic condition is without effect when the time that elapses between its administration and the test dose is less than 18 hours.

II. *What is the earliest time after the inoculation of the nucleoprotein at which evidence of protection against living infection is noticeable?*

A. Using toxic nucleoprotein in 0·01 mg. doses, *i.e.* $\frac{1}{10}$ lethal dose:

1 day	after the inoculation	8	died	16	survived,	*i.e.* 66 %	survived
2 days	,, ,,	2	,,	18	,, ,,	88	,,
3 ,,	,, ,,	0	,,	17	,, ,,	100	,,
4 ,,	,, ,,	2	,,	17	,, ,,	89	,,
14 ,,	,, ,,	2	,,	17	,, ,,	89	,,

B. Using the relatively atoxic nucleoprotein in 0·01 mg. doses, *i.e.* $\frac{1}{100}$ lethal dose:

1 day	after the inoculation	8	died	11	survived,	*i.e.* 60 %	survived
2 days	,, ,,	7	,,	11	,, ,,	72	,,
3 ,,	,, ,,	3	,,	14	,, ,,	88	,,
4 ,,	,, ,,	4	,,	15	,, ,,	84	,,
14 ,,	,, ,,	2	,,	12	,, ,,	83	,,

The results of these experiments may be put in the form of a curve when their meaning becomes more evident.

From these curves the following conclusions are justified:

1. That after the inoculation of nucleoprotein in 0·01 mg. doses the development of the resulting immunity is very rapid, being distinctly evident 24 hours after vaccination[1].

2. The highest point on the curve, *i.e.* the greatest immunity, occurs on the third day.

3. That a slightly better immunity is evident on the third day after the inoculation of the more toxic vaccine.

The conclusion that for the rapid production of immunity it is advantageous to employ a toxic vaccine is capable of control in the following way. In previous reports it was pointed out (vol. XI. supplement, p. 36) that the toxicity of the nucleoprotein was greater the shorter the time elapsing since its extraction from the bacillus, and that there was reason to suppose that as it existed within the body of the bacillus its toxicity was at a maximum. Consequently if there is any relation between the toxicity and the rapidity of onset of the immunity, the vaccination by the same dose of nucleoprotein wrapped up in the

[1] Working with Haffkine's prophylactic as an immunising dose, wild Indian *Mus rattus* and an emulsion of a plague rat's spleen as an infecting dose, Stevenson and Kapadia (*Report of the Bombay Bacteriological Laboratory for* 1911, p. 26) found evidence of some protection as soon as 8½ hours after vaccination.

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bodies of the bacilli should be followed by a still earlier development of the maximum immunity. Using such doses of whole bacilli killed by heat (about 0·1 mg.) as contained 0·01 mg. of nucleoprotein, the following results were obtained:

1 day after the inoculation	18	survived	1	died,	*i.e.*	95	%	survived
2 days	,,	,,	16	,,	3	,,	,,	84	,,
3 ,,	,,	,,	14	,,	3	,,	,,	82	,,

These results are plotted in figure 1.

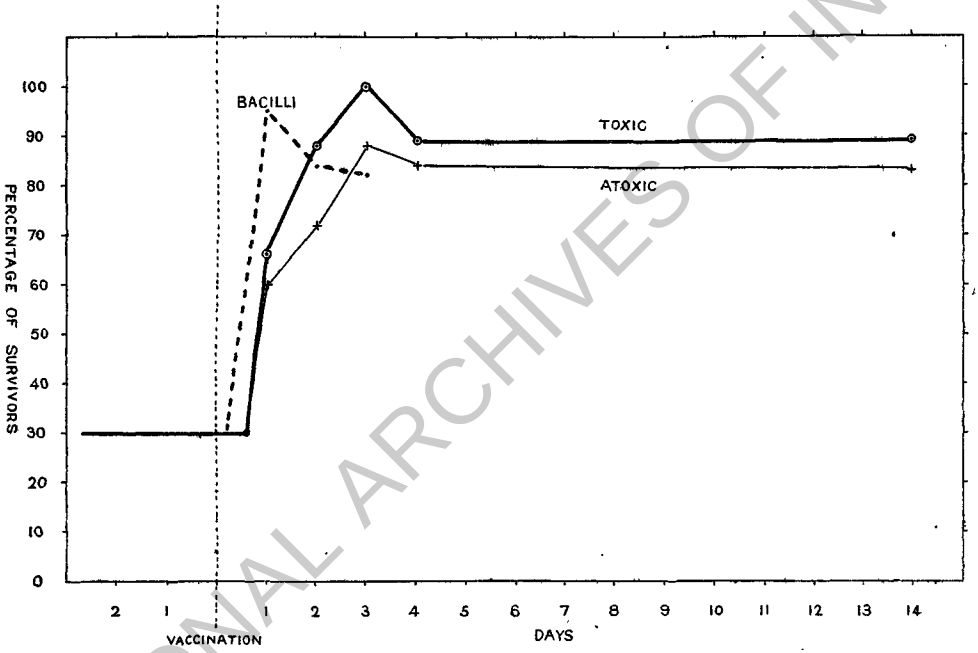

Fig. 1.

Considering the three curves together it will be seen that the highest point is reached first in the case of the whole bacillus vaccine, that is in the case of the vaccine in which we have reason to suppose that the contained nucleoprotein is in the most toxic condition. The conclusion was previously (vol. XI. supplement, p. 42) reached that the protective and toxic properties of the nucleoprotein contained in the plague bacillus were distinct. This conclusion was based on the consideration of the results of vaccination of rats when the interval between the vaccinating and the test dose was 14 days. At this time it remains true that there is no discoverable difference in the results of

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the two (toxic and relatively atoxic) vaccines. But in the earlier stages of the onset of immunity a difference emerges. With the more toxic vaccine the onset of immunity is slightly more rapid; this early rise however appears to be associated with a corresponding early fall so that at the end of a fortnight there is no difference in the height of the immunity curve.

An observation of some interest was made in connection with the series of rats last quoted. These rats were inoculated (vaccinated) with whole bacilli that had been heated to 60° C. for half an hour. The resulting immunity was completely established as early as the first day. With a view to determining the fate of the bacilli used as the vaccine, certain of the rats were kept and the site of vaccination examined from day to day. There was no difficulty in recognising the presence of the bodies of these bacilli. Now it has been repeatedly shown that all the immunity that a culture of plague is capable of conferring on rats is represented in the nucleoprotein extract obtained from them by the methods described in these reports, the rest of the bacilli being incapable of conferring any immunity. In conformity with this, in the series under consideration the bodies of the bacilli were recognisable microscopically up to the fourth day after the inoculation, that is three days after the establishment of all the immunity that the vaccinating dose of organisms is capable of conferring. The resulting immunity must therefore have depended on the escape of the contents of the bacilli, the presence of any unabsorbed portion being hardly capable of being considered responsible.

The duration of the immunity.

Already, on the fourth day after the vaccinating dose of nucleoprotein the differences observed in the height of the immunity curve according as fresh or autolysed nucleoprotein has been used for the vaccination are seen to be very small. After the expiration of 14 days these differences are no longer significant.

It is noteworthy however that the height of the curve remains constant after the slight drop between the third and fourth day, that is to say that the immunity once established remains at the same level. It will now be shown that this level is maintained for some months. The three curves being so nearly coincident at the expiry of 14 days the question of the differences in the extent of the immunity following the inoculation of the nucleoprotein in its fresh or autolysed condition will not be further considered.

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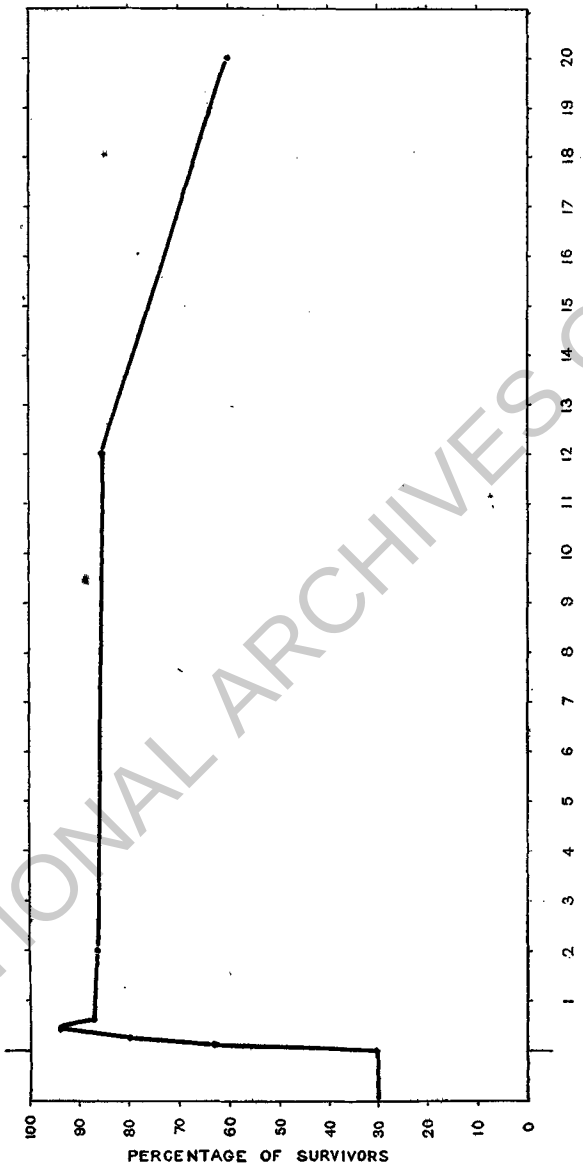

Fig. 2.

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In connection with these experiments considerable difficulty has been encountered in keeping rats alive and healthy for long periods of time. It was hoped that the curve of immunity might be continued for a year at least. On two occasions some hundreds of rats were vaccinated and provision made for keeping them for a long period. This has not been found possible. Epidemics of various diseases broke out during the hot summer of 1911, and after the disappointing loss of much valuable material it has only been found possible to obtain reliable data up to five months after the vaccination (see fig. 2).

At three months after vaccination a series that received 0·1 mg. and one that received 0·01 mg. are available. Of the first series every one of 19 rats survived the test inoculation. Of the second 85 % survived, the series comprising 20 rats. The immunity thus lasts unimpaired for three months at the same height as it was at the end of 14 days.

At five months only one series of 31 rats was available. Of these 18 survived the test dose, *i.e.* 60 %, from which it would appear that in rats the immunity after a single dose of the nucleoprotein is still very appreciable after five months though a good deal less than after three months.

It need hardly be pointed out that the times ascertained in these experiments for the onset and duration of immunity in the rat cannot be directly transferred to any other species of animal, especially when the size and rate of metabolism are widely different from those obtaining in the rat.

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LXII. THE OPSONIC INDEX IN PLAGUE VACCINATION.

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(*From the Lister Institute of Preventive Medicine.*)

In this paper an attempt is made to correlate the production of immunity in rats vaccinated against plague with the production of immune opsonin in the serum of such animals.

Various antibodies appear in the serum of animals inoculated with plague vaccines, but the agglutinins and bactericidal substances, for example, do not lend themselves to satisfactory quantitative study in the case of plague. The opsonic response has been studied in man by Douglas[1] who found a good response after inoculation with Haffkine's prophylactic. It, therefore, seemed advisable to correlate the development of opsonin with the absolute immunity of some susceptible animal in order to see whether the amount of opsonin produced could be used as evidence of the development of immunity in man.

In most of the following experiments the results obtained with a single dose of vaccine are recorded in relation to the opsonic index: in others the results of repeated dosage are shown. These results should be considered in the light of actual immunity experiments with test lethal doses of plague, as performed on vaccinated animals by S. Rowland and described above (p. 367).

The scope of the enquiry resolved itself into the following points:

1. Is there any correlation between immunity and opsonin-production in plague infection?
2. What is the influence of previous vaccination in the experimental animal?
3. What is the source of opsonin in immune plague serum?

Methods of experiment.

In the following series of experiments the dilution method of Klien (1907) was adopted.

[1] Communicated to the Pathological Society of London, 1906.

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In high concentrations of immune serum phagocytosis of plague bacilli is obscured by (*a*) digestion of the bacilli inside the leucocytes, and (*b*) by clumping of bacilli. By means of the dilution method these difficulties are overcome. The ordinary method of incubating the opsonic mixtures in capillary pipettes did not give satisfactory results as the bacilli were found to precipitate and form clumps during the process, thus giving very uneven counts in smears from the same mixtures. I found the most satisfactory results were obtained by making the opsonic mixtures in small tubes which were subsequently placed in a shaking machine in a 37° C. incubator. The mixtures were incubated for a period of twenty minutes.

Throughout the period of experiment a strain of avirulent plague bacillus (K. 120) was used in the opsonic technique, and in vaccinations with the whole bacillus. The nucleoprotein of the plague bacillus used for vaccination was also prepared from the same source. The cultural characteristics of this bacillus have been given by Rowland (vol. x. p. 563).

The bacterial emulsion was prepared each day from a twenty-four hour agar slope, and throughout the period of experiment agar from the same batch was used. This fact is of some importance as it has been observed that great alterations in the phagocytic index may be caused by using bacilli grown on a fresh batch of agar during the time of experiment (Noon, 1909). The cultures were emulsified in 5 % formalin solution and placed in the hot room (37° C.) for one hour in order to kill the bacilli. This was regarded as a necessary precaution on account of the danger in spreading slides containing live bacilli. In one case a control with live bacilli was made which showed that the employment of formalised cultures did not appreciably alter the phagocytic index. The ordinary methods of obtaining fine emulsions, such as shaking with glass beads, do not give good enough emulsions of plague bacilli for opsonic work. To get over this difficulty the following method was adopted. The killed formalin emulsions were decanted into small centrifuge tubes and spun down for a few minutes. The supernatant fluid was then poured off and the bacterial residue was thoroughly broken up by means of a small sable-hair brush, normal saline solution was added and the process repeated two or three times. Finally a short centrifuging was given to precipitate any remaining clumps, the top layers of the emulsion removed to a separate tube and then thoroughly mixed with a capillary pipette. Emulsions of approximately uniform turbidity were used throughout the experiments.

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The pooled serum of ten rats was used for each experiment. The first bleeding was performed before inoculation. The rats were vaccinated subcutaneously in the thigh and were bled from the tails, a couple of drops of blood being taken from each animal. The blood was allowed to clot out over night at room temperature and the serum was separated each morning, and used for opsonic determination the same day. The serum dilutions were prepared immediately before the daily opsonic determinations.

Throughout the experiments I used preparations of my own leucocytes, prepared in the usual manner. The emulsions were used about one hour after preparation in order to give the leucocytes time to recover from any paralysis induced by centrifugalisation, but were not allowed to remain unused long enough to allow any deterioration of activity to set in. One hundred leucocytes were counted on each slide. Broken leucocytes and leucocytes in clumps of more than three were rejected in counting.

In each of the opsonic mixtures one volume of normal saline, one volume of serum dilution, one volume of bacterial emulsion and one volume of leucocytes were used. The counts of the falling dilutions were compared with a saline control in each case and controls of normal rat serum were from time to time employed[1].

Materials used as vaccine.

The specific nucleoprotein employed was made by Dr Rowland in the manner described by him (Rowland, 1908). The powder "B" employed represents the bodies of the bacilli, with their contained nucleoprotein, in a mixture of anhydrous sodium sulphate. The number of grams of the powder used was added to twice the quantity of distilled water at 37° C. and the mixture was then filtered at 37° C. through a Büchner filter. The residue was scraped from the filter-paper and emulsified in distilled water and after centrifuging down a portion of the supernatant fluid was set aside for estimation of nucleoprotein. The nucleoprotein in this portion was precipitated by boiling after the addition of acetic acid and the precipitate carefully collected, washed, dried and weighed in the usual way. The amount of nucleoprotein in the original solution was thus controlled and the necessary dilutions made therefrom.

[1] With normal rat serum an opsonic effect was found in serum dilutions of $\frac{1}{16}$ and $\frac{1}{32}$ but never in higher dilutions than these.

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When the whole bacillus was employed as a vaccine, the surface growth of six Roux bottles, incubated for five days and killed by heating for half an hour at 60° C., was swept off into 5 c.c. normal saline and thoroughly shaken up with glass beads in a stout flask. Three c.c. of this emulsion were then centrifuged down in a tared centrifuge tube and after several washings with distilled water, the residue was evaporated in a hot-air oven at 105° C. till the weight was constant. The remainder of the emulsion was then diluted down to the required strength. Although plague nucleoprotein is readily reduced in toxic and immunising properties by heating, Rowland has shown that this does not obtain so long as the nucleoprotein remains in the bodies of the bacilli (Rowland, 1911).

The slides containing the opsonic films were fixed with methyl alcohol and stained with dilute Giemsa stain for fifteen minutes.

A. Experiments on rats.

Experiment I.

Each of a series of ten rats was inoculated with $\frac{1}{100}$ mg. of freshly prepared nucleoprotein solution in 1 c.c. normal saline. The animals were bled before inoculation, ten hours later and subsequently every morning. The following table gives the results of opsonic counts from day to day.

	1	2	3	4	5	6	7
Serum dilution	Before inoculation	10 hours	22 hours	2 days	4 days	5 days	18 days
1/16	2·53	—	—	—	—	—	—
1/32	1·10	—	—	—	—	—	—
1/64	1·05	—	—	—	—	—	—
1/128	—	—	—	—	—	—	2·05
1/256	—	2·52	—	—	2·10	1·00	1·70
1/512	—	1·96	1·66	—	2·00	1·62	0·98
1/1024	—	1·80	1·58	1·50	1·02	1·49	0·91
1/2048	—	1·80	1·46	1·37	0·92	1·07	—
1/4096	—	1·00	1·73	·87	0·93	1·01	—
1/8192	—	0·98	1·10	·87	—	—	—
1/16384	—	—	1·02	—	—	—	—
1/32768	—	—	1·02	—	—	—	—
Normal saline control	1	1	1	1	1	1	1

Taking the highest dilution of serum which produces an effect greater than that of normal saline, the following result is obtained.

Before inoculation	10 hours	22 hours	2 days	4 days	5 days	18 days
1/16	1/2048	1/4096	1/2048	1/512	1/1024	1/256

The curve obtained by plotting out these figures is shown on chart I.

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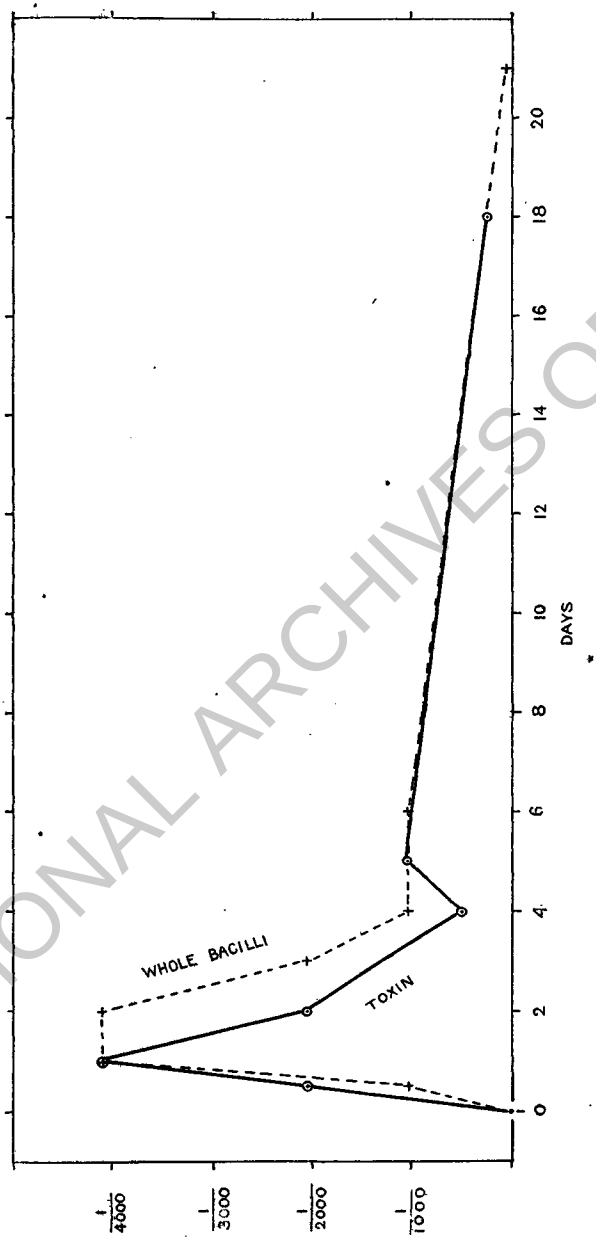

Fig. 1.

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It will be seen from this that the maximum is obtained within 24 hours and afterwards the curve steadily falls so that on the eighteenth day a serum dilution of $\frac{1}{512}$ corresponds to the saline control. It has been shown that full immunity to a lethal dose of virulent plague is obtained within the same period, thus showing that, for this dose of vaccine, the production of opsonin follows the immunity reaction, at least in the early period of immunity.

Experiment II.

Ten rats were inoculated with $\frac{1}{10}$ mg. of whole bacillus vaccine. Rowland has shown that the nucleoprotein content of the bacillus equals about $\frac{1}{10}$ by weight of the whole bacillus, consequently this dose of whole vaccine is comparable to the dose of derived vaccine in experiment I.

The following results were obtained in this series, and the plotted curve obtained therefrom is also shown on chart I.

Before inoculation	10 hours	22 hours	2 days	3 days	4 days	6 days	21 days
1/16	1/1024	1/4096	1/4096	1/2048	1/1024	1/1024	1/64

The results obtained are similar to those obtained in experiment I. The optimum of the curve is displaced slightly towards the right. This difference is so slight that one would not be inclined to place too much significance on the fact, which, however, might possibly be due to the fact that the nucleoprotein in the second case takes an appreciable time to soak out of the bodies of the bacilli.

The general conclusions from these experiments would seem to show, as found by S. Rowland by directly determining the protection aroused in rats to a subsequent fatal dose of living plague, that the soluble nucleoprotein contained in the plague bacillus is the responsible agent in the production of opsonin.

Experiment III.

Ten rats were each inoculated with a very small dose of nucleoprotein solution, *i.e.* $\frac{1}{12000}$ mg. in 1 c.c. normal saline. At the end of ten hours samples of blood were taken but no opsonic response was observed. The curve commenced to rise the next morning and reached its maximum on the third day after which it gradually fell.

Before inoculation	10 hours	22 hours	2 days	3 days	4 days	5 days	6 days	8 days
—	—	1/512	1/1024	1/2048	1/2048	1/1024	1/828	1/512

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Compared with the curves obtained with the larger doses in experiments I and II, it will be observed that with this extremely small quantity the maximum is postponed to the right and that the curve does not rise to anything like the same height.

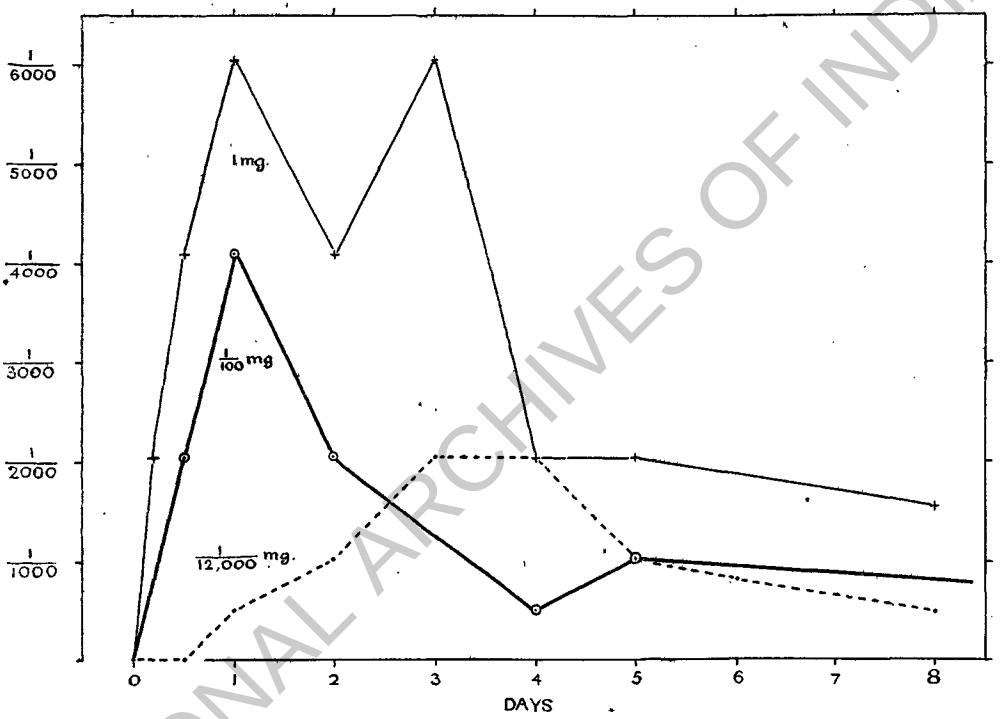

Fig. 2.

Experiment IV.

The ten rats in this series were given a very large dose of nucleoprotein, *i.e.* 1 mg. in 1 c.c. saline. In fresh solution this dose is usually fatal to rats, so the solution was incubated for 48 hours at 37° C. under toluol in order to allow a certain amount of hydrolysis to take place, which Rowland has shown reduces toxicity without interfering with the protective value of the nucleoprotein. In spite of this preliminary hydrolysis, four rats died during the experiment with symptoms of acute plague toxaemia.

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The rats were bled 1 hr., 5 hrs., 10 hrs., 22 hrs., and subsequently every morning, after inoculation.

Before inoculation	1 hour	5 hours	10 hours	22 hours	2 days	3 days	4 days	5 days	8 days
—	—	1/2048	1/4096	1/6044	1/4096	1/6044	1/2048	1/2048	1/1536

The curve obtained in this experiment is higher than in the foregoing series, but this increase is not commensurate with the increased dose of vaccine given to the animals. A point has apparently been reached when increased dosage of vaccine does not bring about a proportional increase in opsonin production, as the amount of opsonin present is not much greater than with a tenth of the dose, as given in experiment I.

The drop in the curve after the sixth bleeding is remarkable and may be attributed possibly to the fact that two of the rats bled at this time died shortly afterwards of toxaemia. The curve rose after this, and the general tendency of the curve is towards a maintenance of the maximum for a longer period than in series I and II.

Experiment V.

Series II, which received a dose of $\frac{1}{10}$ mg. of whole vaccine, was given a repeated dose of the same quantity ($\frac{1}{10}$ mg.) thirty-five days later, when the index had been normal for some weeks. The following results were obtained.

Before inoculation	10 hours	1 day	2 days	3 days	4 days	7 days
—	1/4096	1/16384	1/24476	1/24476	1/16384	1/4096

It will be observed that in comparison with the curves obtained in series II, that the ordinates of the curve are very much higher than those obtained in the latter series, also that the optimum point is postponed to the right, taking place between the second and third days instead of between the first and second days.

Rats vaccinated with this dose retain their immunity for about a year. The opsonin production falls in the first week after inoculation, but the animals are still quite sensitive to a fresh dose of vaccine, and greatly increased response is elicited on re-vaccination.

Experiment VI.

In this experiment the washed bodies of the bacilli were employed for inoculation. The nucleoprotein was extracted in the usual way and the residue was washed and centrifuged down daily. This suspension

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of bacterial bodies was kept under toluol to prevent decomposition. At the end of five days the supernatant fluid was quite free from precipitable nucleoprotein.

Each of a series of ten rats was inoculated with 0·9 mg. of these extracted bodies corresponding to a dose of 0·1 mg. whole vaccine and to 0·01 mg. of nucleoprotein. The serum of these animals was tested from day to day, but no increase in opsonic index was obtained throughout the experiment.

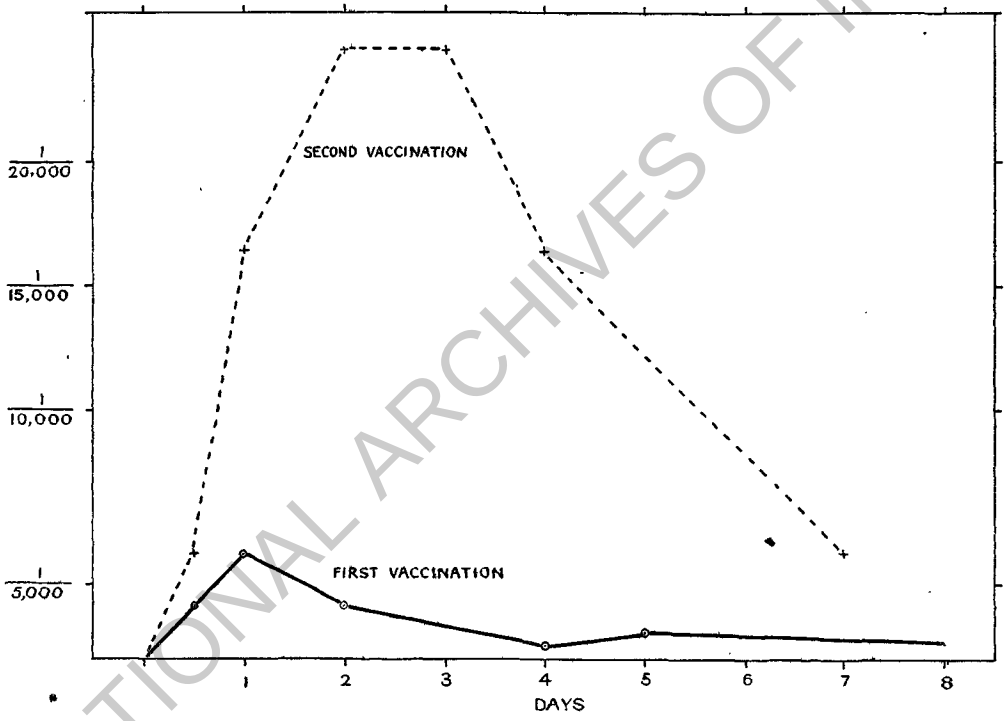

Fig. 3.

Summary of rat experiments.

From the study of the foregoing experiments the following facts appear to emerge. The production of opsonin in immune plague serum at first rises with the production of immunity, and soon returns again to normal leaving the resistance to infection (as ascertained by the direct test) still very high (see fig. 4). A second vaccination shows however that the capacity for response is much enhanced. In the

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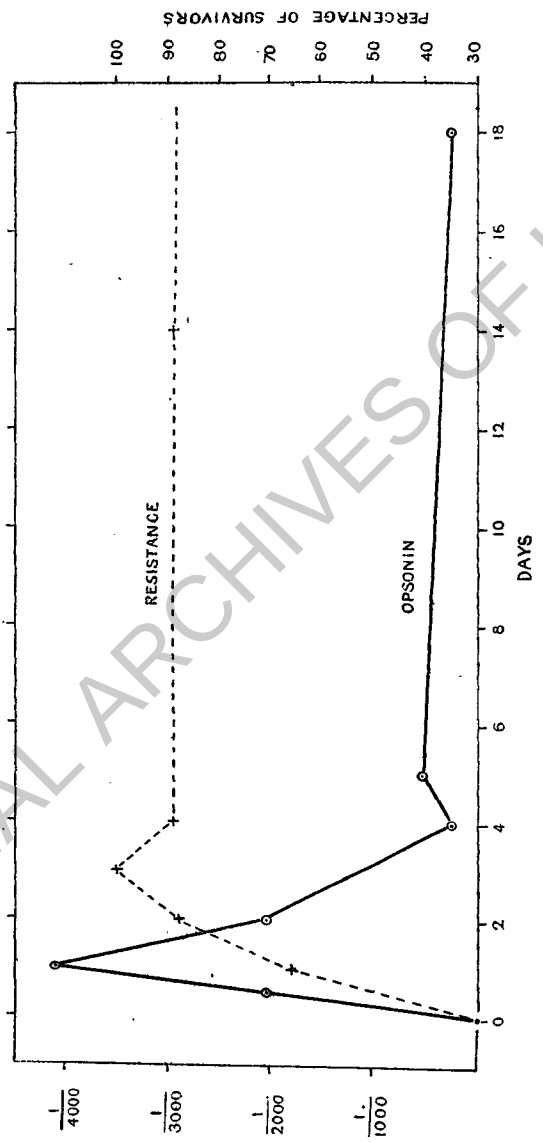

Fig. 4.

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curves representing experiments I and II the maxima of the curves are reached during the first or second day by which time full immunity is established. In the case of the very small dose ($\frac{1}{12000}$ mg.) in experiment III the maximum is postponed till the third day, and with the large dose (1 mg.) in experiment IV, the maximum is maintained for a larger period. The relative quantity of opsonin present is greater with the larger dose of vaccine used for inoculation.

In plague vaccination the active principle producing opsonic rise in the serum of rats resides in the nucleoprotein contained in the bacillus. This is shown (1) by the fact that the curves obtained by use of nucleoprotein solution and whole bacillus respectively are practically the same both as regards date of maxima and height of curve, and (2) by the fact that the washed bodies of the bacilli from which all the nucleoprotein has been extracted have no opsonin-producing value.

B. Human experiments.

Experiment I.

I received $\frac{1}{10}$ mg. nucleoprotein in 1 c.c. saline, which nucleoprotein had been hydrolysing under toluol for five days[1]. This solution was first tested on rats with the following results:

2 rats given ·2 mg. each alive after 1 week.
,, ,, ·4 ,, ,, ,,
,, ,, ·6 ,, ,, ,,
,, ,, 1·0 ,, died in one and three days respectively with post-mortem evidence of plague toxaemia.

The arm at the seat of inoculation was somewhat stiff and painful for 48 hours. I suffered from a slight headache next morning but pulse and temperature were normal throughout.

The following results were obtained:

24 hours	2 days	3 days	4 days	5 days	6 days	7 days	9 days
—	—	1/1024	1/768	1/1024	1/1536	1/512	1/512

It will be observed that the maximum point of the curve in this case is very much later than in any of the curves obtained with rats, being on the sixth day instead of the first to third days.

Experiment II.

The pooled serum of five persons who had been at some time previously inoculated with Haffkine's prophylactic and who had been

[1] This dose of nucleoprotein represents the amount of nucleoprotein contained in 1 c.c. of Haffkine's prophylactic.

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given an inoculation of nucleoprotein solution by me was tested on eight successive days for opsonin after inoculation. The nucleoprotein solution was the same as used in the last experiment, but had been hydrolysing for 12 days.

Initials	Symptoms	Last dose of Haffkine's prophylactic
J. W.	Slight stiffness in arm and dizziness next morning, pulse and temperature normal.	About 12 months
C. C.	Slight stiffness in arm next morning, pulse and temperature normal.	,,
S. R.	No symptoms, pulse and temperature normal.	Over 12 months.
I. R.	Slight stiffness in arm, pulse and temperature normal.	10 months.
F. W.	No symptoms, pulse and temperature normal.	About 8 months.

The following results were obtained with the pooled serum:

1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days
—	—	1/512	1/512	1/1024	1/1024	1/512	1/512	1/256

It will be observed that the top of the curve is reached between the fifth and sixth days after which it gradually falls. No increase of opsonin was observed until the third day. The five persons inoculated were practically without symptoms throughout the experiment.

Experiment III.

The pooled serum of five persons who had never been previously inoculated with plague vaccine and who had been given a dose of $\frac{1}{10}$ mg. hydrolysed nucleoprotein, subcutaneously, were tested for opsonin. The nucleoprotein solution had been hydrolysing for eleven days. A dose of 1 mg. was non-lethal for rats.

Initials	Symptoms
H. D.	Slight erythema at seat of inoculation, pulse and temperature normal.
H. P.	,, ,, ,, ,, ,,
A. S.	No symptoms.
C. F.	,,
G. M.	,,

Before inoculation	3 days	4 days	5 days	6 days	7 days	10 days
Less than 1/32	1/128	1/128	1/256	1/384	1/256	1/192

In this series the opsonic response was not as great as in series II. The number of persons in each series is small and one would hesitate to draw any definite conclusion from these results, but as far as they go they tend to show that pre-existing sensibility has an influence on the opsonic index in human plague vaccination.

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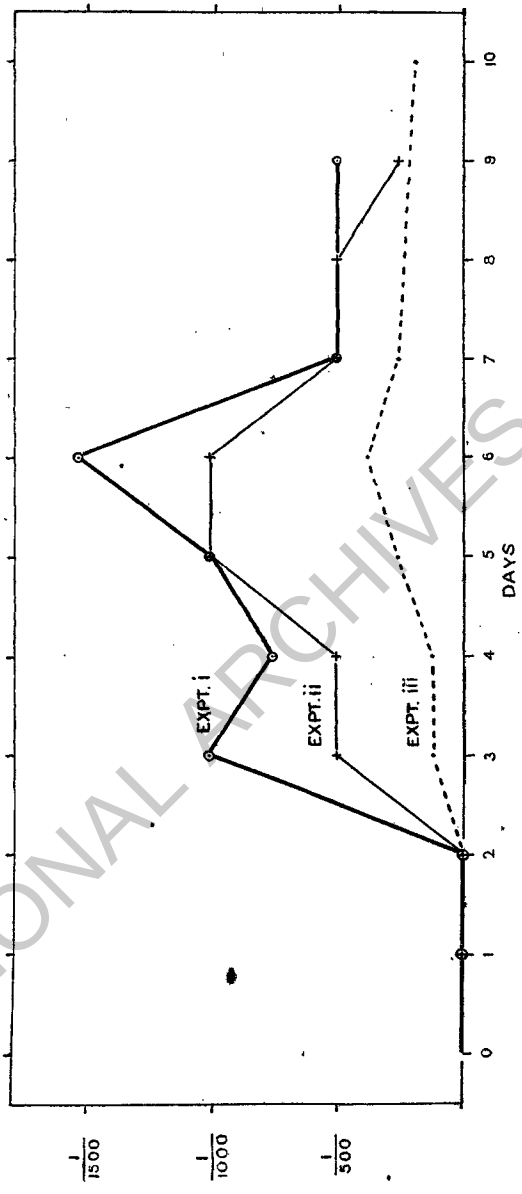

Fig. 5.

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Conclusions.

1. The substance which produces a rise in the opsonic index in immune plague serum is the nucleoprotein contained in the bodies of the bacilli.

2. The washed bodies of the bacilli when used as a vaccine do not cause any increase in the opsonic index.

3. Within limits, the larger the dose, the greater the opsonic response.

4. A second dose of vaccine raises the index above the maximum of the first inoculation, even after the curve has fallen to its normal level.

5. In the early stages, in rats, the opsonic response and the degree of protection aroused rise together.

6. In human beings the maximum response is observed to be much later than in the case of laboratory rats, and the response in a series of previously vaccinated persons is somewhat greater than in a series of persons not previously vaccinated.

7. The local and constitutional effects of nucleoprotein vaccination compare very favourably with those observed in other methods of protective inoculation.

I desire to express my thanks to Prof. C. J. Martin, F.R.S., Director of the Lister Institute, and to Dr J. C. G. Ledingham, Chief Bacteriologist, Lister Institute, for valuable advice and suggestions throughout the inquiry, and particularly to Dr Sydney Rowland, without whose constant advice and help the experiments could not have been carried out.

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LXIII. THE PREPARATION OF ANTITOXIC PLAGUE SERA.

By A. T. MacConkey, M.B., D.P.H.

Bacteriologist in charge of the Serum Department of the Lister Institute.

That it is possible to obtain an antitoxic as distinguished from an anti-infectious plague serum was shown by Markl (1898, 1901, 1903) and other workers. The strongest serums have been those obtained by Dean (1906) and Rowland (1911). The former used for the immunisation of horses the filtrates from old (8—10 weeks) bouillon cultures and obtained a serum of which 1 c.cm. neutralised either 150 or 450 M.L.D. of the toxin according as one takes the death-time-limit of the M.L.D. to be 48 hours or 4 days. Rowland used a toxin extracted from the plague bacillus by means of his "sulphate process" and the serum so obtained was found to neutralise about 100 M.L.D. per c.cm.—the death-time-limit of the M.L.D. being 48 hours. In order to compare these two serums we must base our calculations on the 48 hour limit, and judging by this standard there is not much to choose between them. This 48 hour death-time-limit is the most convenient one to take as the *majority* of rats which have received an injection of plague toxin either die within 48 hours or live longer than 7 days. For this reason it was decided to take this limit in the following experiments and *for the purposes of comparison* to consider any animal alive on the 3rd day as cured of the intoxication due to the plague toxin. For therapeutic purposes a serum which kept animals alive for 3 days only would of course be useless, but experiments made with such a serum might well point out the road to the attainment of better results.

It seems advisable to call attention here to the fact that occasionally many rats which survived the acute attack and which had at the end of a week apparently quite recovered from the inoculation of the toxin-serum mixture, died later in a condition of marasmus. This may be due either to the failure on the part of the serum to neutralise some constituent of the toxin which comes into play after

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the acute attack has passed off or, as some results suggest at first sight, to the serum itself possessing toxic properties. For example, the following mixtures of toxin and antitoxin were made (the volume in each case being made up to 4 c.c.), allowed to stand at room temperature for ¾ hour and then injected into rats subcutaneously.

Calcutta (bleeding 3. 2. 12). Rats 90—95 grms.

2·1 mgm.	toxin	+ 1 c.cm.	serum	= †within 48 hours
,,	,,	+ 0·75	,,	= lived
,,	,,	+ 0·5	,,	= †by morning of 5th day
,,	,,	+ 0·25	,,	= lived
,,	,,	+ 0·1	,,	= lived
,,	,,	+ 0·04	,,	= lived
,,	,,	+ 0·02	,,	= †within 48 hours
,,	,,	+ 0·01	,,	= † ,, ,,

†=died

Now the M.L.D. of this toxin was found to be between 0·56 mgm. and 0·7 mgm. and 2·1 mgm. was considered to be equal to three available minimal lethal doses. We are then faced with the paradox that while 1 c.cm. of serum failed to neutralise 3 M.L.D. this amount of toxin was rendered ineffective by 0·1 c.cm. and 0·04 c.cm.

If this result be due to some toxic property inherent in the serum itself, then large doses of serum alone ought to have a similar effect. But this is not the case as I have injected subcutaneously into rats 10 c.cm. of an antiplague serum, which gave this "paradox-result," without any ill effects. On another occasion 2 c.cm. of three other similar sera were injected subcutaneously into three rats. Two of these rats seemed ill on the second day, but they soon became quite well again. So it cannot be said that the serum itself is toxic in the doses given.

Besides what I have for convenience termed the "paradox-result," there is another source of confusion in evaluating a serum. It has been shown by Rowland (1910, p. 544) that a certain dose of his virulent plague culture will when injected subcutaneously into rats kill on an average only 70 out of 100, *i.e.* with regard to this particular dose some 30% of rats are immune. As there is this natural immunity to infection, it is allowable to presume that the opposite condition may also occur, and that some rats may be more markedly susceptible than others to the influence of plague toxin. It is to these causes that are due the irregularities which not infrequently appear in the tests.

The result of the following experiment may be taken as showing the existence of an increased susceptibility,

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0·553 mgm. toxin + 4 c.cm. normal rat serum = lived
,, ,, ,, + 3 ,, ,, ,, = died
,, ,, ,, + 2 ,, ,, ,, = lived
,, ,, ,, + 1 ,, ,, ,, = ,,

while the opposite condition, namely one of immunity, appears to be present in the case of the first rat in the series below:

1·66 mgm. toxin + 1 c.cm. normal rat serum = lived
0·88 ,, ,, + ,, ,, ,, ,, = died
0·76 ,, ,, + ,, ,, ,, ,, = ,,

A good example of irregularities due to the causes above mentioned is furnished by the tests of a sample of serum from the horse *Lemberg*.

Lemberg. 10. 4. 12.

1*st test* 11. 4. 12. Rats weighing 95—110 grm. Inoculations subcutaneous.
2·1 mgm. toxin (3—4 M.L.D.'s) + 1/60 c.cm. serum = lived
,, ,, + 1/80 ,, = † within 24 hours
,, ,, + 1/100 ,, = lived
,, ,, + 1/150 ,, = † within 36 hours

The animals that lived did not show any symptoms of illness and so it was concluded that a repetition of this test would show definitely what was the value of this serum.

2*nd test* 15. 4. 12. Rats weighing 90—100 grm. Inoculations subcutaneous.
2·1 mgm. toxin + 1/60 c.cm. serum = † within 24 hours
,, + 1/80 ,, = † ,,
,, + 1/100 ,, = † ,,
,, + 1/150 ,, = † ,,

It is obvious that this second test does not help us. We can only infer either that the rats in the second test were hypersusceptible or that two of the rats in the first test were immune. So it was necessary to test this serum a third time.

3*rd test* 18. 4. 12. Rats weighing 90—120 grm. Inoculations subcutaneous.
2·1 mgm. toxin + 1/20 c.cm. serum = lived
,, + 1/30 ,, = ,,
,, + 1/40 ,, = ,,
,, + 1/50 ,, = ,,
,, + 1/60 ,, = † within 36 hours

We may now conclude that the antitoxic value of this serum is represented in the above test by $\frac{1}{50}$ c.cm. and that consequently 1 c.cm. = 150 M.L.D.

An endeavour was made to obtain in the case of each sample tested a clean result such as that given above, but it was not possible to attain this end in every case. In those cases where a result was obtained

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similar to that of the first of the above tests the serum would be valued at $\frac{1}{60}$th c.cm. or 180 M.L.D. per c.cm.; when the result was like that of the second test one could only say that the serum did not neutralise 180 M.L.D.'s per c.cm.

The next point to be considered is the toxin used as a test toxin. I have used various products as follows:

(1) *filtrates* of broth culture are rarely toxic enough and are highly unstable. Thus in 29 trials, only twice was a filtrate obtained of which 0·1 c.cm. would kill a rat; in one instance a filtrate, which, when first tested, killed a mouse of 25 grammes within 24 hours in a dose of $\frac{1}{100}$th c.cm. subcutaneously, had no effect three days later on a mouse of the same weight in a dose of $\frac{1}{15}$th c.cm.

(2) *Solutions of the toxic nucleoproteid* give good results when used fresh, but they lose toxicity steadily (vol. XI. supplement, p. 35); ultimately they seem to become relatively stable, but the results then obtained with them are quite different from those obtained with the same serum against fresh toxin solution, and call to mind the suggestion that there may be more than one toxic substance in the plague bacillus.

(3) *A dry preparation of the toxic nucleoproteid* has also been tried but gave irregular results in the serum tests.

(4) The most constant preparation which I have tried has been obtained by taking a quantity of the dry mass formed by mixing the bacilli with anhydrous sodium sulphate, melting it by placing it in the incubator at 37° C., obtaining an even distribution of the bacilli by thorough stirring and shaking and then solidifying the mixture quickly by pouring it into a shallow basin cooled by ice. This "toxin rock" (TRk 1) may be broken up into small pieces and kept in a stoppered bottle in the dark. When required for use, a small quantity is weighed out, dissolved in 0·85% NaCl at 36° C., cooled to about 0° C. and filtered cold[1].

In the experiments detailed below

"*fresh toxin*" = a freshly prepared solution of toxic nucleoproteid,
"*SR* 2" = an old autolysed solution of the same,
"*SRNP* 1" = the toxic nucleoproteid in the form of a dry powder,
"*TRk* 1" = the "rock" of sulphated bacilli.

In the following table are given the results of a series of tests of the same serum with various toxins.

[1] A second sample of this "rock" did not give such satisfactorily constant results.

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Serum Y 83. *Wealdstone* bleeding 28. 1. 11.

Date when tested	Toxin used as test toxin	No. of rat minimal lethal doses neutralized per c.cm. of serum
13. 4. 11	Bouillon filtrate 7 c.	500
28. 6. 11	SR 2	< 50
1. 7. 11	Fresh toxin	50 ? +
4. 7. 11	SR 2	30
13. 10. 11	,,	< 2
16. 10. 11	Fresh toxin	160
28. 11. 11	SR NP 1	? 10 ? 0
1. 12. 11	,,	? 20 ? 0
,,	Fresh toxin	600
30. 1. 12	TRk 1	60 +
1. 2. 12	,,	150

With the idea of bringing out the differences more plainly the details of one or two of these tests are given below.

Serum Y 83. Tested 1. 12. 11. Toxin SR NP 1. Rats weighing 90—105 grms.

Serum. 3 mgm. toxin + 1 c.cm. serum = † within 18 hours
,, +0·5 ,, = † ,, 48 ,,
,, +0·25 ,, = † ,, 48 ,,
,, +0·1 ,, = † ,, 6th day
,, +0·05 ,, = lived

Controls. 0·75 mgm. toxin = lived
0·5 ,, ,, = † within 48 hours

NOTE. The M. L. D. of this toxin usually lay between 0·75 mgm. and 0·5 mgm.

Serum Y 83. Tested 1. 12. 11. Toxin = fresh toxin. Rats weighing 85—120 grms.

Serum. 3 mgm. toxin + 1 c.cm. serum = † within 18 hours
,, +0·5 ,, = lived
,, +0·25 ,, = † **5th day**
,, +0·1 ,, = lived
,, +0·05 ,, = † within 18 hours

Controls. 0·75 mgm. toxin = † within 18 hours
0·5 ,, = † ,, ,,
0·25 ,, = † ,, ,,
0·1 ,, = † 48—60 hours
0·05 ,, = † within 48 hours

Inoculations in each case were made subcutaneously. Toxin and serum in contact for $\frac{3}{4}$ hour at room temperature before injection.

The tests of this serum performed on 30. 1. 12 and on 1. 2. 12 using as test toxin "toxin rock" TRk 1 were of quite a different order:—

30. 1. 12. Rats weighing 90 grms.

2·1 mgm. toxin + 1 c.cm. serum Y 83 = no symptoms, lived
,, +0·5 ,, ,, = ,, ,,
,, +0·25 ,, ,, = ,, ,,
,, +0·1 ,, ,, = ,, ,,
,, +0·05 ,, ,, = ,, ,,

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1. 2. 12. Rats weighing 90—115 grms.

2·1 mgm. toxin + 0·05 c.cm. serum = no symptoms, lived
,, + 0·02 ,, = ill, lived
,, + 0·01 ,, = † within 48 hours
,, + 0·005 ,, = † ,, ,,

NOTE. The M.L.D. of toxin TRk 1 lay between 0·7 mgm. and 0·56 mgm. and nearer the latter than the former.

It is clear that toxin rock TRk 1 gave the cleanest results and as it acted similarly with other serums it was taken into use as a test toxin. We see also from the tests of Y 83 that neither the autolysed toxin SR 2 nor the toxic nucleoprotein powder SRNP 1 are suitable to be used as test toxins. That filtrates of bouillon cultures, which are in reality solutions of autolysed toxin, should give better results than a toxin such as SR 2 is peculiar, but is interesting when considered in connection with Kolle's statement (1903, p. 361) that neither Bern nor Paris serum nor the serum produced by the injection of centrifuged old bouillon cultures had any neutralising effect upon the toxin contained in old bouillon cultures, and that he could not confirm Markl's statement that it is possible to produce an antitoxin against the poison of old cultures. This behaviour of toxin SR 2 is very puzzling and recalls the suggestion that there may be two toxins produced by the plague bacillus, and that this may be the same kind of toxin as is spoken of by Kolle. Or Palandino-Blandini's (1905) statement may apply to toxin as well as cultures. He says that typhoid and plague serum protect against higher multiples of the M.L.D. when virulent strains are used than when weakened strains are used, because in the last case a larger comparative amount of bacterio-nuclein is injected and the bacterio-nuclein influences the protective power of the serum. This can only apply if there is a total destruction of toxin. But if we consider the test made on 4. 7. 11 another possible explanation is forthcoming. The details of that test are as follows:

Y 83 serum. Toxin SR 2. Rats weighing 90—95 grms.

3 c.c. toxin SR 2 + 1·0 c.c. serum = no symptoms, lived
,, ,, + 0·5 ,, = ,, ,,
,, ,, + 0·25 ,, = ill but recovered
,, ,, + 0·1 ,, = ,, ,,
,, ,, + 0·05 ,, = † within 24 hours

Serum and toxin in contact for $\frac{3}{4}$ hour before injection.

This is a perfectly clean result and we can say definitely that 0·1 c.cm. of serum neutralized 3 c.cm. of toxin. Now at the time of the test the M.L.D. of this toxin was 0·75 c.cm. or a little less, but not

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0·5 c.cm., and the test dose would contain 4 M.L.D. of which we can only count three as available for evaluating the serum, so that 1 c.cm. of serum would neutralise 30 M.L.D. But the original M.L.D. of this toxin was about 0·2 c.cm. which would make the test dose of 3 c.cm. equal to 15 M.L.D., and as this was neutralised by 0·1 c.cm. of serum the serum would be per c.cm. equivalent to 150 original M.L.D. This titre is the same as the serum had when tested against toxin TRk 1 which was an unmodified toxin.

This is rather against the existence of two separate toxins and also against an absolute destruction of toxin and suggests that, as in the case of diphtheria toxin, the plague toxin has become modified into "toxoid"—some $\frac{3}{4}$ of each original M.L.D. having been thus converted.

Such a toxin, *i.e.* one that has become modified by being allowed to undergo autolysis, is capable of producing immunity and antitoxin as is shown by the case of the horse *Bombardier* which was immunised with comparatively non-toxic toxin. When first prepared this toxin had an M.L.D. for rats of about 0·2 mgm., but after being kept for 4–6 weeks at 36° C. the M.L.D. had risen to about 3 mgm. The details of the immunisation are given below. The toxin was injected into the muscles of the neck just in front of the scapula.

Immunisation of Bombardier.

Date	Mgms. of toxin inoculated	No. of rat M.L.D.'s inoculated	Sample bleeding	Date of test	Toxin used as test	No. of rat M.L.D. neutralised per c.cm.
May 4, 1911	10	3—4	—	—	—	—
,, 16 ,,	20	7	—	—	—	—
,, 26 ,,	40	14	—	—	—	—
June 9 ,,	80	28	—	—	—	—
July 4 ,,	150	50	—	—	—	—
,, 13 ,,	300	100	—	—	—	—
,, 14 ,,	—	—	20 c.cm.	—	—	—
,, 20 ,,	—	—	,,	20. 7. 11	SR 2	5
,, 27 ,,	600	200	—	—	—	—
,, 28 ,,	—	—	20 c.cm.	28. 7. 11	SR 2	5
,, 30 ,,	—	—	,,	31. 7. 11	,,	5
Aug. 1 ,,	—	—	,,	1. 8. 11	,,	5
,, 3 ,,	—	—	,,	4. 8. 11	,,	5
,, 4 ,,	520	170	—	—	—	—
,, 9 ,,	1000	333	—	—	—	—
,, 16 ,,	—	—	20 c.cm.	16. 8. 11	SR 2	30

From a study of this table we learn several things. Firstly we see that injections of this modified toxin can give rise to the production of antitoxin. The amount of antitoxin was at first quite as large and

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finally much larger than that usually found in the serum of horses immunised according to Yersin's method, *i.e.* intravenous inoculations of living cultures.

Secondly, we see that the introduction of 600 mgm. in divided doses produced an antitoxin content which was not increased when 600 mgm. was given in one dose after an interval of 14 days, but that 1520 mgm. given in two doses with only a five-day interval increased the amount of antitoxin in the blood six times.

Lastly we see that the quantity of antitoxin in the blood was the same 24 hours after as it was before inoculation, and was found to be still at the same level when tested on the third, fifth and seventh day after injection. If this modified toxin caused a "negative phase" one would expect to find evidence of it 24 hours after the inoculation of such a dose as 600 mgm. (the growth from 171 Roux bottles). We may conclude that there is in this case either no negative phase or one with a duration of less than 24 hours. Now if such a large dose causes only a short negative phase or none at all, it is justifiable to presume that a small dose, such as would be used as a prophylactic measure, would not have any ill effect and consequently that such a modified toxin would be eminently suitable for use as a "vaccine."

It will have been noticed that during the immunisation of *Bombardier* samples of blood were taken at intervals of 24 hours, 3 days, 5 days and 7 days after the injection of 600 mgm. of modified toxin, and that the antitoxin content of the serum was the same on each occasion. The question then naturally arises "Does this condition obtain after doses of other toxins and at what time after inoculation does the serum contain the greatest quantity of antitoxin? When horses are immunised by means of intravenous injections of living cultures, it is usual to bleed about three weeks after the last injection, but the reasons for choosing such a late date in the case of living cultures need not necessarily hold good when toxins are used. So a series of samples of blood were taken at intervals of 7, 14 and 21 days after an injection and the serum tested for antitoxin. The results are given in the following table.

From a consideration of the table one would draw the conclusion that the antitoxin content of the blood does not vary much between the 6th and the 21st day. This condition proved most convenient as it enabled one to space the doses nicely and at the same time to take samples to ascertain the effect of each dose—thus a sample could be taken and then a dose given at weekly intervals.

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Name of Horse	No. of rat M.L.D. neutralised per c.cm. of serum 7 days	14 days	21 days	Remarks
Lemberg	100 *	<120 †	<120 †	* Tested against bouillon filtrate 7 c. † ,, ,, fresh toxin
,,	9	9	9	Tested against toxin SR 2
Whiteface	30	30	30	,, ,, ,,
Calcutta	30	30	30	,, ,, ,,
,,	30	? 30	30	,, ,, ,,
,,	75	75	—	,, ,, TRk 1
,,	750	525 (12 days)	—	,, ,, ,,
Wealdstone	30 (6 days)	? 30 (13 days)	60 (20 days)	,, ,, SR 2
,,	75	50	—	,, ,, TRk 1
,,	225	240 (12 days)	—	,, ,, ,,

Proceeding in this manner with three horses which had already been immunised the results embodied in the following table were obtained:

Horse	No. of rat M.L.D. neutralised per c.cm. of serum Before immunisation	7 days after 10 mgm.	7 days after 25 mgm.	7 days after 50 mgm.	7 days after 75 mgm.	14 days after 75 mgm.
Wealdstone	12	12	30	75	75	50
Calcutta	60	60	12	30	75	75
Lemberg	30	30	30	30	75	75

NOTE. The toxin used for immunisation was freshly prepared before each injection and the size of the dose is given in milligrammes of nucleoprotein as calculated from an analysis of a similar powder of "sulphated bacilli." The doses must be considered not as absolutely accurate but only approximate, for there are always slight differences in the nucleoprotein content of different batches of powder.

It appears that in the case of an immunised horse, which is resting after a bleeding, one inoculation of freshly prepared toxin has not much effect upon the antitoxin content of the blood—as obtains also in the case of diphtheria and possibly of other toxins. The effect of a 2nd, 3rd and 4th dose is somewhat variable when each dose is considered separately, but the summation effect is to stimulate the production of antitoxin.

The amount of antitoxin production in these three horses in consequence of the injection of 112 mgm. of fresh toxin was not great, and so they were given a rest and then inoculated with an autolysed toxin. From the experience gained with *Bombardier*, it was decided to try the effect of giving the doses at intervals of three days instead of a week but as the reaction following the second dose had not passed off by the third day, the third dose was given seven days after the second, and a sample taken to ascertain the effect of the first two doses.

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Samples were also taken at various intervals after the third dose. The antitoxin content of these serums is given in tabular form below.

Horse	No. of rat M.L.D. neutralised per c.cm. of serum								
	8 days before inoculation	7 days after 100 and 200 mgm.	7 days after 300 mgm.	12 days after 300 mgm.	19 days after 300 mgm.	24 days after 300 mgm.	32 days after 300 mgm.	30 days after 300 mgm.	46 days after 300 mgm.
Wealdstone	50	150	225	240	300	—	300	90	90
Calcutta	75	300	750	525	360	450	300	300	120
Lemberg	75	210	300	300	450	—	240	150	150

NOTE. In the case of Lemberg owing to scarcity of toxin the third dose was only 100 mgm. instead of 300 mgm. as in the case of the other two horses.

We see that, just as in the case of *Bombardier*, doses of autolysed toxin at short intervals have produced a marked rise in the antitoxin content of the serum. The horses were bled 8 litres on the 12th day after the last injection, and in each case a slight improvement in the serum took place 7–12 days after bleeding. After that a gradual decrease occurred in the amount of antitoxin in the blood, until on the 46th day after the last injection the strength of the serum was only about double what it was before this series of injections was begun. On this date re-immunisation was commenced with a solution of toxin which had been kept at 36° C. for 1 month. This solution was made up to contain 1 mgm. of nucleoprotein per c.cm., and when fresh the M.L.D. for rats was about 0·7 mgm. After a month at 30° C. the M.L.D. had increased to 3 mgm. or 4 times as much as it was originally. The doses given were 30 mgm., 100 mgm. and 300 mgm., corresponding respectively to about 43, 148 and 430 original M.L.D. or 10, 33 and 100 M.L.D. of the autolysed toxin. The results were

Horse	No. of rat M.L.D. neutralised per c.cm. of serum			
	Before re-immunisation	3 days after 30 mgm.	7 days after 100 mgm.	9 days after 300 mgm.
Wealdstone	90	150	300	240
Calcutta	120	180	300	300
Lemberg	150	150	<180	240
Kitasato	60	60	60	90

Here again we see that the response to autolysed toxin is better than to fresh toxin. Of course it may be said that the doses of fresh toxin were not so large nor given in such a short time as those of the autolysed toxin, and that therefore the results are not comparable. Such criticism is quite fair and one would have liked to have avoided it, but from my experience I should say that such doses of fresh toxin

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could not be injected at such short intervals because the reaction following inoculation would be too great to allow of its being done. But we may compare the toxins not according to volume in c.cms. or weight in mgms., but according to the number of M.L.D. given. If we value the toxins according to their M.L.D. at the time of inoculation there is not so very much difference between the doses. In the first series the total number of M.L.D. was about 160, in the second about 200 and in the third about 144 M.L.D. If however we value them according to the M.L.D. when freshly prepared the differences are great, and the respective numbers would be about 160, 850 and 600 M.L.D. The difference in the amounts of antigen given in the above three series of inoculations may possibly be made more striking if we calculate the quantity of growth which was required to produce the nucleoprotein. Rowland's analyses have shown that on an average the growth from 1 Roux bottle yields 3·5 mgm. of nucleoprotein, and taking this amount as the basis of our calculations we find that the number of Roux bottles used was

Horse	1st series	2nd series	3rd series
Wealdstone	47	198	123
Calcutta	47	198	123
Lemberg	47	132	123

and the results suggest that other things being equal the response to an inoculation is more dependent on the amount than on the absolute toxicity of the antigen introduced. This agrees with the opinion expressed by Kretz (1909, p. 26), that the effect of the toxin in the production of antitoxin (diphtheria) depends not so much on its direct toxic value as on its antitoxin neutralising power—old weakened toxin giving as good results as fresh toxin.

Besides the toxicity and amount of antigen given there is another factor which influences the result. As in the cases of diphtheria and of tetanus so in the case of plague, each horse does not respond alike to a given stimulus. This is well illustrated by the results of the immunisations of the two horses *Ramsay* and *Wealdstone*.

Ramsay had between the 20th July 1910, and the 19th October 1910, 1340 mgm. of *fresh* toxin equal to about 4000 rat M.L.D. or the growth from some 380 Roux bottles, and yielded a serum of which 1 c.cm. neutralised some 400 rat M.L.D. *Wealdstone* had the first injection on Aug. 24, 1910, and by Oct. 10 had received 200 mgm. of fresh toxin. On Oct. 19th the serum had a titre of 150 M.L.D. per c.cm. The immunisation was continued, and by Jan. 7, 1911, the total quantity of toxin introduced was 3000 mgm. or 9000 rat M.L.D. or the

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growth from some 860 Roux bottles. On Jan. 28, 1911, the titre of the serum was per c.cm. 500 M.L.D. when tested against a toxic bouillon filtrate, but only some 150 M.L.D. when tested against "fresh toxin." For our present purpose we will take the larger of these two values and comparing it with that of *Ramsay* we see that doubling the amount of antigen has only raised the titre of the serum by a fraction and that therefore the "individuality" of the horse has a great deal to do with the response which follows the introduction of the plague antigen. *Wealdstone* had still larger doses of toxin—1000 mgm. 2000 mgm., 2000 mgm. and 3000 mgm. in single doses without any resulting increase in the titre of the serum.

We came therefore to the conclusion that it was useless to increase the size of the doses of fresh toxin to any great extent, not only because they seemed to be ineffectual in increasing the antitoxin content, but also because the local and constitutional reactions were so great that the intervals between the inoculations had to be lengthened from the 4 or 5 days usual at the commencement to one month at the end.

Both fresh and old toxin, but particularly the former, when given in doses of 100 mgm. and upwards cause a loss of flesh which is not to be accounted for by the temporary loss of appetite and the febrile condition brought about by the injection.

To sum up we may say that in the case of horses which have been already immunised, old weakened toxin appears to stimulate the production of antitoxin better than fresh toxin, and it has the great advantage of not interfering so much with the well-being of the animal, cf. Pane, 1911, p. 279.

In these tests the toxin and serum have been mixed and allowed to remain in contact for about $\frac{3}{4}$ hour at room temperature before injection into the rats. Other workers however have used a higher temperature and allowed a shorter or a longer time for union to take place, and so experiments were made to ascertain the effect of these two factors. Toxin and serum were mixed, the volume when necessary made up to about 4 c.cm., and the mixture allowed to stand at 36° C. for $\frac{3}{4}$ hour and for $1\frac{1}{2}$ hours. Control doses of toxin alone were also diluted and kept at 36° C. for the same time. There was no difference in the results following the injection of mixtures or pure toxin kept at room temperature or at 36° C. for $\frac{3}{4}$ hour or for $1\frac{1}{2}$ hours.

The next point considered was whether other antisera possessed the power of neutralising plague toxin to any great extent. For this purpose the following tests were performed.

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Various antisera.

1 c.cm. of serum was mixed with decreasing amounts of plague toxin, made up to about 4 c.cm. when necessary, allowed to remain at room temperature for ¾ hour and injected subcutaneously into rats. The M.L.D. of the toxin used was between 0·7 mgm. and 0·56 mgm. The numbers show the weights of the rats in grammes.

Name of horse	Variety of serum	Date of bleeding	Date of experiment	Amounts of toxin 2·1 mgm.	1·4 mgm.	0·7 mgm.	0·56 mgm.
Tasmania, *Melbourne*, *Adelaide*, *Hobart*, *Wellington*	Pooled serum of 5 normal horses	28. 6. 11; 29. 6. 11; 29. 6. 11; 29. 6. 11; 29. 6. 11	12. 2. 12	+ 90	+120	+120	lived 120
Bangor	Antitetanic serum	9. 1. 12	12. 2. 12	+ 90	+ 90	+ 90	+ 90
Vignette	Had had injections of living streptococci and also of diphtheria toxin	19. 1. 12	12. 2. 12	+ 90	+ 95	lived 90	lived 90
Maude	Antistreptococcic serum	1. 11. 11	12. 2. 12	+ 90	+ 90	lived 90	lived 95
Arkwright	Anti pseudotubercle serum	10. 6. 8	19. 2. 12	+100	+ 90	+ 90	lived 90
Broncho	Antidysentery (intravenous inoculations)	26. 7. 6	28. 2. 12	+105	+110	+100	+100
Nobby	Antidysentery (intramuscular inoculations)	28. 2. 12	4. 4. 12	+105	lived 120	lived 120	lived 115
,,	Antidysentery (intramuscular inoculations)	29. 3. 12	4. 4. 12	+120	lived 125	lived 120	lived 120
Rich	Antistaphylococcic	21. 2. 07	28. 2. 12	+ 90	+100	+100	lived 105
Whitestreak	Antidiphtheritic (1300 units)	8. 2. 12	28. 2. 12	+115	+115	+100	lived 105
White Lady	,, (150 units)	12. 1. 11	28. 2. 12	+120	+120	lived 120	+120

Antiplague serum.

2·1 mgm. of plague toxin were mixed with decreasing amounts of antiplague serum, made up to 4 c.cm. where necessary, allowed to remain in contact ¾ hour at room temperature and then injected into rats subcutaneously.

Name of horse	Date of bleeding	Date of test	Amounts of serum 1 c.cm.	0·5	0·25	0·1	0·02	0·01
**Corbally*	22. 1. 09	28. 2. 12	lived 90	lived 100	lived 110	† 110	—	—
**Whiteface*	15. 2. 09	23. 2. 12	,,	lived 95	† 90	† 90	—	—
† ,,	26. 2. 12	26. 2. 12	,,	lived 90	lived 90	lived 90	—	—
Ramsay	5. 11. 10	28. 2. 12	—	—	—	—	lived 115	lived 90
Wealdstone	14. 3. 12	20. 3. 12	—	—	—	—	lived 90	† 100

* Immunised with living cultures given intravenously.

† ,, ,, filtrates intramuscularly.

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From these results we are justified in concluding that neither normal serum nor any of the ordinary antisera are capable of neutralising plague toxin to any appreciable extent compared with anti-plague serum.

Having determined that the antitoxin in antiplague serum is a specific antitoxin two questions naturally occur to one. Does it retain its antitoxic power for any length of time unimpaired? What effect will tropical temperatures have upon it?

As regards these questions Chouroupoff (1909) examined the protective and curative properties of ordinary antiplague Yersin serum of various ages up to 10 and 11 years. He found the 11 year old serum useless and thought the loss of power might be due to the 0·5% phenol present. From his experiments he concluded that if serum be heated at 55° C. on each of three successive days for 1½ hours and then be kept in the cold, in the dark and in the absence of air it will preserve its curative and protective properties unchanged for six years. Wladimiroff (1898) states that antiplague serum keeps up its strength for months and is not affected by passing through the tropics. Our tests do not go back far enough to enable us to speak of years, but we have the results of tests performed by Rowland and myself with an interval of some 18 months to 2 years between them.

The results are given in the following table:

Name of horse		Method of immunisation	Date of bleeding	Tested in 1910 by S.R. 1 c.cm. serum =	Tested in 1912 by A.T.M.C. 1 c.cm. serum =
Corbally	Y 65	Living cultures intravenously	22. 1. 9	10 M. L. D.	12 M. L. D.
Whiteface	Y 68	,, ,, ,,	15. 2. 9	5—10 ,,	6 not 12 M. L. D.
Kitasato	Y 72	,, ,, ,,	16. 12. 9	,, ,,	12 not 30 ,,
Whiteface	Y 74	L. I. V. + toxin	20. 12. 9	40+ ,,	60 not 90 ,,
Lightfoot	Y 75	Toxin only	29. 12. 9	84 ,,	90 M. L. D.
,,	Y 76	,, ,,	4. 1. 10	125 ,,	30 ,,
,,	Y 77	,, ,,	18. 10. 10	66 ,,	30 not 60 M. L. D.
Ramsay	Y 79	,, ,,	5. 11. 10	450 ,,	300 not 450 ,,

In these tests the M.L.D. of the toxin was about 0·7 mgm. or a little less, and the test dose was 2·1 mgm. The quantities of serum used were in the following descending scale: 1 c.cm., 0·5, 0·25, 0·1, 0·05, 0·02, 0·01 and 0·0066. This will explain why no values are given between 30 and 60 or between 300 and 450. These results show that plague antitoxin retains its value fairly well for 18 months to 2 years.

To ascertain the effect of heating on the antitoxin some small serum vials were filled with antiplague serum, sealed off in the flame and then heated in a water bath for ½ hour at 56° C. on each of three successive days.

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Name of horse	Date of bleeding	Antitoxin content in M.L.D. Before heating	After heating
Wealdstone	3. 2. 12	75 M. L. D. per c.cm.	75 M. L. D. per c.cm.
Calcutta	3. 2. 12	30 ,, ,,	75* ,, ,,
Lemberg	3. 2. 12	30 ,, ,,	30 ,, ,,

* This appears to be an instance of a rat being hyperresistant. In the test before heating the rats which received $\frac{1}{25}$ c.cm., $\frac{1}{50}$ c.cm. and $\frac{1}{100}$ c.cm. of serum all died and in consequence it was not thought to be worth while to test the serum after heating beyond $\frac{1}{25}$ c.cm.

It is obvious that heating antiplague serum under the conditions described has no deteriorating effect upon the neutralising power for plague toxin.

Concentration of antitoxin.

As the titre of the serums obtained has not been very high an attempt was made to increase the value of a serum by the Gibson concentration process which has proved so successful in the case of diphtheria antitoxin. Five hundred cubic centimetres of serum were precipitated with an equal volume of half-saturated solution of ammonium sulphate. The precipitate was collected and extracted with brine. Eleven hundred (1100) c.cm. of brine filtrate were obtained and precipitated with 0·25% of acetic acid. The precipitate was collected, made alkaline with powdered washing soda and dialysed against tap water. When found comparatively free from salts the contents of the dialyser—200 c.cm.—were filtered first through chain cloth and then through a small Berkefeld filter candle.

The antitoxic values at the three stages were:

Original serum	500 c.cm.	90 M. L. D. per c.cm.
Brine extract	1100 ,,	45 ,, ,,
*Concentrated serum	200 ,,	150 ,, ,,

* Unfortunately the serum became contaminated in the dialyser and perhaps some of the loss may have been due to this cause.

There is thus an increase in the antitoxic value per c.cm. to the extent of $1\frac{2}{3}$, but on the whole a loss of 33% of antitoxin.

SUMMARY.

1. It is possible to obtain a plague antitoxic serum by means of injections of toxin obtained from the plague bacillus by Rowland's sulphate process. So far however the antitoxic value has not been high. The usual neutralising power has been 300—400 rat M. L. D. per c.cm. and on only one occasion was a value of 750 M. L. D. reached.

2. In an immunised horse one obtains a better response, as regards antitoxin production, if one uses an old weakened toxin instead of a fresh toxin.

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3. Plague serum retains its antitoxic properties for months unimpaired.

4. Heating at 56° C. for ½ hour on each of three successive days has no effect on the antitoxin.

5. Plague antitoxin may be concentrated by the same process that is used for concentrating diphtheria antitoxin.

I desire to take this opportunity of thanking my colleague Dr S. R. Rowland for so generously placing at my disposal the large amounts of toxin which have been required. To my chief, Dr C. J. Martin, I am greatly indebted for much kindly and helpful criticism.

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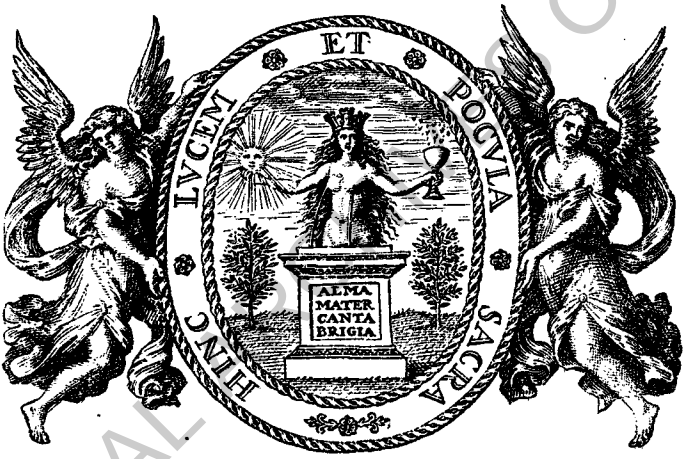
THE
CAMBRIDGE UNIVERSITY PRESS
BULLETIN

No. XXIV October XX, MCMXI



Notes on books published at the
Cambridge University Press
Fetter Lane, London, E.C.
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The Cambridge Medieval History. Volume I, The Christian Roman Empire and the Foundation of the Teutonic Kingdoms. *Planned by J. B. Bury, M.A., Regius Professor of Modern History. Edited by H. M. Gwatkin, M.A., and J. P. Whitney, B.D.*

Royal 8vo. pp. xxii+754. With a portfolio of 14 maps. Price 20*s.* net.

Extract from the Preface

The present volume covers a space of about two hundred years beginning with Constantine and stopping a little short of Justinian. At its opening the Roman Empire is standing in its ancient majesty, drawing new strength from the reforms of Diocletian and the statesmanship of Constantine: at its close the Empire has vanished from the West, while the East is slowly recovering from the pressure of the barbarians in the fifth century, and gathering strength for Justinian's wars of conquest. At its opening heathenism is still a mighty power, society is built up on heathen pride of class, and Rome still seems the centre of the world: at its ending we see Christianity supreme, Constantinople the seat of power, and the old heathen order of society in the West dissolving in the confusion of barbarian devastations. At its opening Caesar's will is law from the Atlantic to Armenia: at its ending a great system of Teutonic and Arian kingdoms in the West has just been grievously shaken by the conversion of the Franks from heathenism direct to orthodoxy.

In our first chapter we trace the rise of Constantine, his reunion of the Empire, his conversion to Christianity, the political side of the Nicene Council, and the foundation of Constantinople. Then follows Dr Reid's account of the reforms of Diocletian and Constantine, which fixed for centuries the general outline of the administration. After this Mr Norman Baynes takes up the struggle with Persia under Constantius and Julian, and continues in a later chapter the story of the wars of Rome in East and West in the times of Valentinian and Theodosius. The victory of Christianity is

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treated by Principal Lindsay; and he describes also the rival systems of Neoplatonism and Mithraism, and gives an account of Julian's reaction and the last struggles of heathenism. The next chapter is devoted to Arianism. First the doctrine is described, in itself and in some of its relations to modern thought; then the religious side of the Nicene Council is given, and the complicated history of the reaction is traced down to the decisive overthrow of Arianism in the Empire by Theodosius. After this Mr C. H. Turner describes the organisation of the Church—clergy, creeds and worship—looking back to the beginning, but chiefly concerned with its development in the age of the great Councils.

We now pass to the Teutons. Dr Martin Bang begins in prehistoric times, describing their migrations and their conquests westward and southward till the legions brought them to a stand on the Rhine and the Danube, and their long struggle of four centuries to break through the Roman frontier before the battle of Hadrianople settled them inside the Danube. Then Dr Manitius carries down the story through the administrations of Theodosius and Stilicho to the great collapse—the passing of the Rhine, the overrunning of Gaul and Spain, the Roman mutiny at Pavia, and the sack of Rome by Alaric. After this the great Teutonic peoples have to be dealt with severally. Dr Ludwig Schmidt begins with the settlement of the Visigoths in Gaul, traces the growth and culmination of their kingdom of Toulouse, and ends with their expulsion from Aquitaine by Clovis. Professor Pfister gives the early history of the Franks; but they are still a feeble folk when he leaves them, for the conquests of Clovis belong to another volume. Then Dr Schmidt tells the little that is known of the Sueves and Alans in Spain, and more fully describes the history and institutions of the Vandal kingdom in Africa to its destruction by Belisarius.

Our next chapter differs from the rest in containing very little history. It is Dr Peisker's account of Central Asia and the Altaian mounted nomads. It is given as a general, and much needed, introduction to the chapters on the Huns, the Avars, the Turks, and the rest of the Asiatic hordes who devastated Europe in the middle ages. To this is attached Dr Schmidt's short account of the Huns and Attila. We

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next turn to our own country. Professor Haverfield describes the conquest and organisation of Roman Britain, and the decline and fall of the Roman power in the island, while Mr Beck deals with the English in their continental home, and tells the story of their settlement in Britain from the English side. After this Mr Barker records the last struggles of the Western Empire—the loyalty of Gaul and the disaffection of Africa—under Aëtius and Majorian, concluding with the barbarian mutiny at Pavia which overthrew the last Augustus of the West. Then M. Maurice Dumoulin continues the history of Italy under the barbarian rule of Odovacar and Theodoric, describing the great king's policy, and shewing how he kept in check for awhile the feud of Roman and barbarian which had wrecked the Western Empire. Turning now to the Eastern provinces, the fifth century, which falls to Mr Brooks, is upon the whole a prosaic period of second-rate rulers and dire financial strain. Yet even here we have striking events, remarkable characters, and important movements—the fall of Rufinus and the failure of Gaïnas; Pulcheria ruling the Empire as a girl of sixteen, the romance of Athenais, and the catastrophe of Basiliscus; the Isaurian policy of Leo, and the reforms of Anastasius. Then Miss Alice Gardner traces the history of religious disunion in the East. The fall of Chrysostom brought to the front the rivalry of Constantinople and Alexandria, the defeat of Nestorianism at Ephesus and of Monophysitism at Chalcedon fixed the lines of orthodoxy, but left Egypt and Syria heterodox and disaffected, and the reconciling Henoticon of Zeno produced nothing but a new schism. In the next chapter Dom Butler traces the growth of monasticism and its various forms in East and West, including the Benedictine rule and the Irish monks. After this Professor Vinogradoff surveys the whole field of social and economic conditions in the declining Empire, and shews the part which rotten economics and bad taxation played in its destruction. Then Mr H. F. Stewart gives his account of the heathen and Christian literature of the time, and of the various lines of thought which seem to converge upon the grand figure of Augustine. The volume concludes with Mr Lethaby's account of the beginnings and early development of Christian art.

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The Constitution and Finance of English, Scottish, and Irish Joint-Stock Companies to 1720. *By William Robert Scott, M.A., D.Phil., Litt.D., Lecturer in Political Economy in the University of St Andrews. Volume III, Water Supply, Postal, Street-lighting, Manufacturing, Banking, Finance, and Insurance Companies; also Statements Relating to the Crown Finances.*

Royal 8vo. pp. xvi+564. With a facsimile and a chart. Price 18*s.* net.

Extract from the Preface

This volume completes Part II of the constitutional and financial history of British joint-stock companies to 1720. In its subject-matter it is differentiated from the second volume, in so far as in the latter most of the undertakings described were related to the shipping industry, whereas those now dealt with were mainly concerned with commerce at home.

Perhaps the addition of the concluding division, which treats of the Crown finances at certain periods, requires some explanation. In this and the second volume references have been necessary to the state of the credit of the Crown; and, owing to the comparative treatment aimed at in Part I, it was essential to allude frequently to these and similar topics. It is unfortunate that there is no modern history of the Revenue, and therefore it became necessary to provide certain illustrative statements of it, which, under the various qualifications mentioned in Division XV, would bridge the gap to some extent. But to have entered on such discussions in detail in Part I would have been out of place, and hence comparatively full tabular statements have been provided in this volume, so that reference from Part I (Vol. I) to these may be as easy as possible to the reader.

In the case of companies which still exist, I am indebted to the officials for information in several directions. The following accounts of such bodies have been read either in MS or in proof by members of the directorate or of the staff who have devoted special attention to the early history of the institutions with which they are connected. In this way, it is to be hoped that accuracy on many minor points has been secured.

NATIONAL ARCHIVES OF INDIA

The Cambridge Modern History. Volume XIII, Genealogical Tables and Lists and General Index. *Planned by the late Lord Acton, LL.D., Regius Professor of Modern History. Edited by A. W. Ward, Litt.D., G. W. Prothero, Litt.D., and Stanley Leathes, M.A.*

Royal 8vo. pp. xii+644. Price 16*s.* net.

The series of *Tables and Lists* supplied in this volume consists, in the first instance, of *Genealogical Tables* of the sovereign families of European and certain other countries, together with *Lists* of elected potentates, within the period treated in *The Cambridge Modern History*. To these are added *Genealogical Tables* of certain noble houses, with *Lists* of chief ministers of great States, and of governors of important dependencies and colonies, within the same period. Finally, *Lists* are given of English, and British, Parliaments and of Imperial Diets, together with one or two lists of a special kind adverted to below.

These *Tables and Lists* are primarily intended as aids in the use of *The Cambridge Modern History*; and no pretence is made that every one of them is complete in itself. It is, however, hoped that they will be found to supply the information likely to be required by a large proportion of students of modern history, and that no personage of any historical importance has been passed by in the table or list where his or her name ought to find a place. The *Tables and Lists* should throughout be used in conjunction with the *General Index*.

Among the *Genealogical Tables* are included those of various families which were prominent in European Succession questions, or took a leading part in the civil and religious wars of the sixteenth and seventeenth centuries. Room has been found for *Tables* of certain other families of which various members are not easily identifiable in the usual books of reference. Thus, several notable Irish clans are given. On the other hand, it has not been thought necessary to include *Tables* of well known houses in the English and the Scottish nobility, which may be traced without difficulty in peerages or other handbooks. The Howard, Douglas, and Campbell families have, however, been exceptionally introduced,

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n order to draw attention to their political alliances; and, on much the same grounds, certain of the great ministerial families of the sixteenth and eighteenth centuries have been included. The Reding *Table*, which has been placed by the side of that of the De Retz family for the sake of convenience only, is intended to show a connexion not usually noticed.

As to order of sequence, the *Tables* and *Lists* have, so far as possible, been arranged under the heads of the countries to which they severally relate, beginning in each division with the British Empire. Here, the governors of the more important colonies only are given, the lists beginning ordinarily from the date when representative or responsible ministerial government was first set on foot in the several colonies.

In the case of the German *Genealogical Tables* selection has not been easy, owing to the large number of sovereign families; so far as possible, preference has been given to those of which prominent members are mentioned in the *History*. The Spanish *Tables* likewise required much compression, owing to the exigencies of space. The identification of individuals is peculiarly difficult here, occasionally in consequence of a form of Spanish pride, which showed itself in the systematic suppression, in family genealogies, of members of the family who had "disgraced" it.

The General Index, which has been for some time in preparation, contains an Index, as complete as possible, of personal and place names occurring in the twelve successive volumes of the *Cambridge Modern History*, the separate Indexes to which have been carefully re-examined, together with summary sequences of the most important events and transactions connected with these names. Entered under the names of the principal countries will be found all the main references to these in the course of the work. Besides the personal and place names, the Index contains certain selected subject headings, intended to furnish additional guidance to the contents of the several volumes. The General Index will thus supply students with facilities of reference extending over an area such as is not at present covered by any other English historical work.

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The Journal of George Fox. *Edited from the MSS by Norman Penney, F.S.A. With an Introduction by T. Edmund Harvey, M.A., Member of Parliament for West Leeds. In two volumes.*

Royal 8vo. pp. Vol. I, xlii+470; Vol. II, viii+530. Price 21*s.* net the two vols.

Extract from the Preface

This edition of *The Journal of George Fox* differs from all its predecessors in that it is printed, *verb. et lit.*, from the original manuscripts, whilst the first edition, which has been followed in all subsequent ones, was much edited and altered, though based upon the same manuscripts.

Permission to print the manuscripts was readily granted by the owner, Robert Spence, of London and North Shields, and students of history will find themselves under great obligation to him and to the Syndics of the Cambridge University Press, for making possible the publication of this Quaker classic in its original form.

The manuscripts were transcribed for the press by J. Guthlac Birch, of London. The printer's proofs have been checked by me with the originals and a few textual notes have been added. It is hoped, therefore, that an accurate representation is now before the reader.....The notes to be found at the close of each volume are the result of a considerable amount of research into many obscure and hitherto unpublished sources.

Extract from the Introduction

To this meeting [of the Quaker ministers and elders] naturally fell the important task of editing for the press the numerous papers which George Fox left behind him, many of which he had expressly desired should be published, and chief among them the "Great Jornall" which he had prepared with the object of giving a faithful record of his public ministry and religious experience.

There can be little doubt that the quiet years from 1675 to 1677 which he spent at Swarthmore Hall were largely utilised for gathering together scattered manuscripts, and for completing up to date this unique religious autobiography,

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which is supposed to have been begun during his last long imprisonment in Worcester Jail in 1673–74, when his son-in-law and fellow prisoner Thomas Lower acted as his scribe. The fact that full notes of Fox's two trials at Lancaster, based on verbatim reports, occur in the *Journal*, corroborates the view that it was compiled in its present form at Swarthmore Hall.

The tradition that the *Journal* was originally dictated in Worcester Jail is supported by the large number of documents relative to George Fox's trial and imprisonment there which are included in the original Manuscript.

It must not be supposed that the *Journal* as we know it from previous printed editions was prepared without much forethought, and extant records show how great was the care taken in its first publication.

The manuscript now reproduced for the first time in its entirety through the action of the authorities of the Cambridge University Press was doubtless regarded by George Fox rather as the rough material than the final form of the work to be printed after his death, but we cannot but be grateful that through the liberality of the Syndics and the loving care of its present editor it is possible for the reader to possess an exact transcript of the original work, with all its errors uncorrected. We are able thus to form an estimate of the character of George Fox and of the history of the Society of Friends during his lifetime which no incomplete edition would give us. Especial importance attaches to the light thrown on the earlier years of the Quaker movement by the new material now published for the first time.

The *Journal* was never intended to be an autobiography in the full sense of the word. Yet if the picture which the *Journal* gives is necessarily incomplete, it is more living and convincing than many a fuller portrait of themselves which other writers have left. As we read its pages there stands out clearly before us the great, strong personality of its writer, with all his shrewdness and simplicity, his untiring devotion to his message and his power of passing it on to others. The prophet's fire, the wise man's counsel, stirring records of hardships bravely borne, quaint and homely touches of human kindness, all are here.

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Educational Charters and Documents 598 *to* 1909.

By Arthur F. Leach, formerly Fellow of All Souls College, Oxford.

Crown 8vo. pp. liv+582. Price 10*s.* net.

Extract from the Introduction

This book aims at doing, so far as the scantier space allows, for the educational history of England what Bishop Stubbs' *Select Charters* did for its constitutional history. It sets out the text of the salient documents relating to the origin and development of educational institutions.

Educational charters, being largely both legal and ecclesiastical, tend to combine the prolixity of the preacher with the verbosity of the conveyancer. Hence, few of them can be presented at full length. As the chief object of the work is to show the origins of educational institutions, which are in many cases centuries earlier than hitherto supposed, the earlier bulk much more largely than the later documents.

In nothing, not even in religion, has the innate conservatism of the human race been more marked than in education. It is hardly an exaggeration to say that the subjects and the methods of education remained the same from the days of Quintilian to the days of Arnold, from the first century to the mid-nineteenth century of the Christian era.

The history of English education begins with the coming of Christianity. But the education introduced by Augustine of Canterbury was identical in means and methods with that of Augustine of Hippo. The conversion of the English caused the establishment in Canterbury of a school on the model of the Grammar and Rhetoric Schools of Rome, themselves the reproduction of the Grammar and Rhetoric Schools of Alexandria and of Athens.

This is brought home to us by the first document in the text, an extract from Bede's *Ecclesiastical History*. It relates how in a year, fixed to 631, Sigebert, King of the East English, with the assistance of bishop Felix, who came from Canterbury, provided masters and ushers after the Canterbury (or Kentish) fashion, and set up a school in which boys might be taught grammar.

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***Dr John Walker and* The Sufferings of the Clergy.** *By G. B. Tatham, M.A., Fellow of Trinity College, Cambridge. The Prince Consort Prize* 1910.

Cambridge Historical Essays. Crown 8vo. pp. viii+430. Price 6*s*.

Extract from the Preface

Walker's *Sufferings of the Clergy* is little known nowadays, and it is probable that of those who are aware of its existence few have had the curiosity to peruse its contents. For the student of the Civil War period, however, it has a considerable interest. It contains a class of information which is not easily found elsewhere, and for an account of the events of the time as they affected individual localities or parishes and, even more, for an account of the clergy themselves, it may still serve as a valuable foundation.....I have attempted to give an account of the circumstances in which the *Sufferings of the Clergy* was written, the materials of which the author was able to avail himself, and the manner in which he used them.

The Theory of Religious Liberty in the Reigns of Charles II and James II. *By H. F. Russell Smith, B.A., St John's College, Cambridge. Thirlwall Dissertation* 1911.

Cambridge Historical Essays. Crown 8vo. pp. viii+144. Price 4*s*.

Extract from the Preface

The period with which I have dealt, suggesting, as it does, the Clarendon Code, the Test Acts, and the Exclusion Bills, is not generally associated with the spirit of tolerance. I have tried to show that, in spite of the contradictory trend of legislation, there was a definite theory of religious liberty, which was asserted from their own points of view by the Nonconformists, the Rational Theologians, and the Whigs. Although it may be true that toleration was given largely from empirical motives, the work of those who prepared the way by forming and popularising the theory must not be underestimated. I have therefore treated toleration on its theoretical side, introducing other aspects only so far as they contributed to the formation of the political theory.

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Extract from the Preface

All we know of Isho'dad is that he was a native of Merv, and became Bishop of Hadatha in Assyria, being much admired for his erudition, wisdom, and splendid personal appearance.....He wrote Commentaries on most of the books of both Testaments. Assemani expressly mentions the Pentateuch, Joshua, Judges, Ruth, Samuel, David (the Psalms), Proverbs, Ecclesiastes, Canticles, and Job, all of which books were supposed by the Syrians to have been translated into their language in the time of Solomon, at the request of his friend Hiram king of Tyre.

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Extract from the Preface

These lectures are intended to serve as a sequel to the course delivered in the University of Aberdeen some ten years previously. If at that time I had foreseen that I should presently be favoured with the opportunity to lecture on the *Realm of Ends or Pluralism and Theism* I might well have entitled the earlier lectures the *Realm of Nature or Naturalism and Agnosticism.* There my endeavour was to establish the priority of the idealistic, or—as it seems clearer to say—the spiritualistic standpoint, and here I have tried to ascertain what we can know or reasonably believe concerning the constitution of the world *interpreted throughout and strictly in terms of Mind.*

At the outset this world immediately confronts us not as one Mind nor even as the manifestation of one but as an objective whole in which we discern many minds in mutual interaction. It is from this pluralistic standpoint that our experience has in fact developed and it is here that we acquire the ideas that eventually lead us beyond it. For pluralism, though empirically warranted, we find defective and unsatisfactory: but the theism to which it points is only an ideal—an ideal however that, as both theoretically and practically rational, may claim our faith though it transcends our knowledge. Such is a meagre outline of the present lectures.

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I wish to record my thanks to various colleagues and friends for their suggestions, many of which I have gladly adopted in preparing this edition,—particularly to Dr W. Brown, Mr T. H. Pear, Prof. Carveth Read, Dr W. H. R. Rivers, Mr Sydney Scott, and Prof. C. Spearman. My gratitude is specially due to Dr H. Head, for having kindly afforded me the opportunity of incorporating into this edition his very remarkable psychological investigations which will later be published in the *Croonian Lectures* delivered by him before the Royal College of Physicians last July.

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The memoirs and vegetation maps published by these workers, of which the titles will be found in the Bibliography on p. 367, have formed the nucleus of the material dealt with in the present book, which is thus a direct outcome of the work of the Central Committee. Its composition would have been impossible but for the close co-operation secured by the existence of such an organisation. Since it was clearly out of the question for a committee as a whole actually to write a book, it was necessary for someone to undertake the task of correlating the contributions of various authors and securing uniformity of treatment. The editor undertook this task on the understanding that he should be allowed a free hand in editing the work of the authors, and this was in nearly every case most willingly accorded. The result of this loyal co-operation has been, it is hoped, to secure a consistent treatment of the whole subject.

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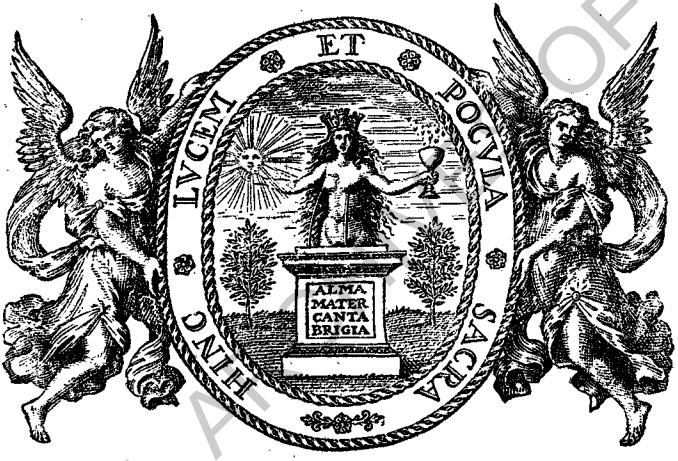

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The Genus Iris. *By William Rickatson Dykes. With forty-seven coloured drawings by F. H. Round, one coloured plate of seeds by Miss R. M. Cardew and thirty line drawings by C. W. Johnson.*

Demy Folio. pp, viii+246. With 48 coloured plates and 30 line drawings. Bound in Roxburgh—dark green leather back and green cloth sides—with lettering in gold and gilt top. Price £6. 6*s*. 0*d*. net.

Publishers' Note

The Genus Iris is the outcome of an attempt to bring together the available information on all the known species of Iris. The account of each begins with the references to it in botanical literature, followed by the references to the various synonyms under which it appears to have been described. A full description of the plant is then given, together with observations on its peculiarities, on its position in the genus, on its value as a garden plant and on its cultivation. These descriptions and notes are based for the most part on living plants, grown in the author's garden, where the majority have been raised from seed. Those species that are not known in cultivation have been described from herbarium specimens. As far as possible the type specimens of each species in the various herbarium collections have been examined and the account of the distribution of each species is based on the results of research in the herbaria of Kew, of the British Museum, of the Botanic Gardens of Oxford, Cambridge, Berlin, Paris, Vienna and St Petersburg and of the United States National Museum at Washington. The various specimens are recorded in detail with dates and the collectors' names. The most striking feature of the book is, perhaps, the inclusion of the **48 life-size coloured plates**, reproduced from originals drawn from living plants. In the text are inserted 30 line sketches of botanical details and of herbarium specimens of species that are unknown in cultivation. Keys are given to the division of the genus into sections and to the species assigned to each; a copious index is also appended.

NATIONAL ARCHIVES OF INDIA

Extract from the Preface

In publishing this book on Irises, I am fully aware that it is not yet possible to give a complete account of the Iris genus. This could only be done by one who had the leisure and the opportunity first of all to go to all the localities in which Irises have been found or in which new species are likely to exist, and then to grow all the species side by side and note their affinities and differences. Meanwhile, this book contains an attempt to put together the available facts and to indicate the gaps in our information. It is hoped that it may lead to the filling up of some of these gaps and to a more general appreciation of the various species of Iris. With regard to the arrangement of the species in groups, it seemed better to take together those plants which are obviously related to one another by their whole appearance, than to pick out some one character or set of characters and base on it an artificial grouping, which would bring together the most widely different species. This plan has made it impossible to give a really satisfactory clavis or key to the Apogon section, but it is hoped that the definitions of the characteristics of the various groups will be a sufficient guide in assigning an Iris to one or other of them.

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Introduction. The Literature of the Iris. Bibliography
The structure, distribution and cultivation of the Iris
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[*A special prospectus of this book, together with a specimen coloured plate, will be forwarded on application*]

NATIONAL ARCHIVES OF INDIA

Herbals: Their Origin and Evolution. *A Chapter in the History of Botany*, 1470–1670. *By Agnes Arber (Mrs E. A. Newell Arber), D.Sc., F.L.S., Fellow of Newnham College, Cambridge, and of University College, London.*

Royal 8vo. pp. xviii+254. With frontispiece, 21 plates, and 113 text-figures. Price 10*s.* 6*d.* net.

Extract from the Preface

The main object of the present book is to trace in outline the evolution of the *printed herbal* in Europe between the years 1470 and 1670, primarily from a botanical, and secondarily from an artistic standpoint....The titles of the principal botanical works, which were published between 1470 and 1670, are given in Appendix I. The book is founded mainly upon a study of the herbals themselves....I have also drawn freely upon the historical and critical literature dealing with the period under consideration, to which full references will be found in Appendix II....The great majority of the illustrations are reproduced from photographs taken directly from the originals by Mr W. Tams of Cambridge, to whom I am greatly indebted.

CONTENTS

The Early History of Botany—The Earliest Printed Herbals (Fifteenth Century)—The Early History of the Herbal in England—The Botanical Renaissance of the Sixteenth and Seventeenth Centuries—The Evolution of the Art of Plant Description—The Evolution of Plant Classification—The Evolution of the Art of Botanical Illustration—The Doctrine of Signatures, and Astrological Botany—Conclusions—Appendix I—Appendix II—Index.

Globe.—Mrs Arber may rest assured that her fragrant volume will be cherished in many a quiet home, both for the sake of its subject and the fascinating manner in which she has treated it....Mrs Arber has succeeded to a remarkable degree in retaining in the reader's mind that indefinable impression of sweetness and perfume which the title of her book brings with it....Of the artistic aspect of the old herbals Mrs Arber has furnished abundant evidence in the liberal fashion in which she has illustrated her book with the woodcuts and plates taken straight from their pages....No one will read it without yielding to the seductive charm which Mrs Arber has contrived so delicately to retain.

[*A special prospectus of this book, including a specimen plate, may be obtained from the publishers on application*]

NATIONAL ARCHIVES OF INDIA

Makers of British Botany. *A Collection of Biographies by living botanists. Edited by F. W. Oliver.*

Demy 8vo. pp. viii + 332. With frontispiece, 26 plates and a text-figure. Price 9*s.* net.

Publishers' Note

The present volume represents in somewhat expanded form a course of lectures arranged by the Board of Studies in Botany of the University of London and delivered during the early part of 1911 in the Botanical Department of University College, London. The ten lectures comprised in the course were delivered by various botanists, the lecturer in each case being either a worker in the same field as, or in some way having a special qualification to deal with, his allotted subject. The seventeen chapters forming the book include these lectures and seven additional chapters.

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Introduction—Robert Morison 1620–1683 and John Ray 1627–1705, by Sydney Howard Vines—Nehemiah Grew 1641–1712, by Agnes Arber—Stephen Hales 1677–1761, by Sir Francis Darwin—John Hill 1716–1775, by T. G. Hill—Robert Brown 1773–1858, by J. B. Farmer—Sir William Hooker 1785–1865, by F. O. Bower—John Stevens Henslow 1796–1861, by George Henslow—John Lindley 1799–1865, by Frederick Keeble—William Griffith 1810–1845, by W. H. Lang—Arthur Henfrey 1819–1859, by F. W. Oliver—William Henry Harvey 1811–1866, by R. Lloyd Praeger—Miles Joseph Berkeley 1803–1889, by George Massee—Sir Joseph Henry Gilbert 1817–1901, by W. B. Bottomley—William Crawford Williamson 1816–1895, by Dukinfield H. Scott—Harry Marshall Ward 1854–1906, by Sir William Thiselton-Dyer—A sketch of the Professors of Botany in Edinburgh from 1670 until 1887, by Isaac Bayley Balfour—Sir Joseph Dalton Hooker 1817–1911, by F. O. Bower—Index.

Aberdeen Journal.—No important aspect of the development of botanical science is omitted; systematic botany, anatomy, physiology, palaeobotany, nuclearcytology, and ecology are each given in proper historical setting, and the survey is both instructive and stimulating.... This is a book which experienced botanists will find not only of genuine interest, but full of suggestiveness with regard to the development of the science, as all good histories are. Further, it is a book to be highly commended to the attention of young botanists, who will not only find the history of their subject pleasantly told, but who will be made to feel the personal spell of those workers most of whose names they are already familiar with, and inspired to enthusiastic effort in their own field.

[*An illustrated prospectus of this book may be obtained on application to the publishers*]

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By Margaret M. Gregson, B.A.

The Cambridge Nature Study Series. Large Crown 8vo. pp. xii + 160. With 75 illustrations. Price 2*s.* 6*d.* net.

Extract from the General Editor's Preface

This book is to help children to study Nature, not to put book study instead of Nature Study. The object of the book is to direct and stimulate both observation and reflection.

Many teachers have lately found in trees a convenient topic for lessons, one that can be pursued in town or country, summer or winter, indoors or out-of-doors. There may be other teachers who would gladly give their pupils some training in Nature Study, although they themselves do not pretend to have special knowledge. Such an acknowledgment of ignorance is itself a qualification for one who, an enquirer himself, would lead others to enquire....

Great pains have been taken in the revision of these chapters to fit them into the natural cycle of the seasons, which is now widely accepted as the best order for nature lessons, and is, indeed, the compulsory order if free use is to be made of fresh material. The supplies necessary for each lesson are clearly indicated. But the lessons have also been adapted (and this is a much more difficult adaptation) to the school year and, again, to the different years in use at different schools....

The serious enquiries now being made into National Afforestation show that we may have to enlist the sympathy of boys and girls in the planting and protection of new woodlands....A chapter on Forestry and tree planting will, therefore, be welcome....

The lessons are adapted to classes where the ages range from 14 to 12 or even younger, that is to the higher standards of elementary schools, to preparatory schools, to the lower forms of secondary schools and especially to those who are taught privately at home.

Athenaeum.—It is a pleasure, among the worthless books published about nature study in its various branches, to come upon Mrs Margaret Gregson's 'Story of our Trees.' Her clear and exact work, and the excellent drawings and photographs by which it is profusely illustrated, should be of great use to the teacher, as well as the pupil.

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Price £2. 2*s.* 0*d.* net.

Extract from the Introduction

The modern artist still lies under the necessity of studying the art of the past. To shut our eyes to it, as some younger ardent spirits would have us do, would mean the extinction of all tradition, and with it of art itself. For all art, and all science, is based on inherited knowledge, and every step onward is made from the last vantage won by those who have gone before us and shown the way....It will therefore be the object of the following pages not merely to describe but to try and explain the development of architecture from style to style since the decline of classic art in the 3rd and 4th centuries of our era, down to the dawn of Gothic architecture, by connecting its constructive details and outward features with those social reasons which served to mould them into the forms we know. From this point of view it is important to compare the rate of progress of the new art in different countries: to mark not only the main current of the movement, but the irregular and unequal advances by which it pushed its way in each instance. For though the general set of the movement was all in one direction it advanced much faster in some places than in others, and in each country it took a distinctive national character. For this purpose the comparative and parallel tables of examples at the end of the book will I hope be found useful. It is important too to observe the continuity of architectural history; how one style gave birth to another; for no new style was ever invented, but always grew out of an older one; how this progression from style to style was always unintentional and unconscious: and how revival after depression always began by the attempt to revive an older art, with the result that when art did revive it was always something new, for no dead art was ever made to live again, or ever will be.

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The Concept of Sin. *By F. R. Tennant, D.D., B.Sc., Author of* The Origin and Propagation of Sin *and of* The Sources of the Doctrines of the Fall and Original Sin.

Crown 8vo. pp. viii+282. Price 4*s.* 6*d.* net.

Methodist Times.—Dr Tennant's new book is accurately described in the title. It is not a formal discussion of sin as one of the main topics of Christian theology, but an attempt to find such a concept of sin as will give an adequate interpretation to facts admitted by all. The definition of sin which is thus arrived at eventually is that it is "moral imperfection for which an agent is, in the sight of God, accountable" (page 245). This concept, it is claimed, is logically perfect, and the only one which can fully satisfy the implications of the most fundamental of Christian doctrines. In addition, it is unimpregnable by psychology, ethics (in the stricter sense), science and history. Not the least interesting and valuable parts of Dr Tennant's work are those in which he examines sin in relation to ignorance, temptation and guilt. If anyone wishes to realise how tremendous a fact sin is, how hard to defeat, how impossible to ignore, and yet how certainly not the ultimate fact in the universe, he cannot do better than study Dr Tennant.

The Northern Whig.—Dr Tennant has read widely on the subject of this volume. But he has done more. He has thought deeply; and it is the utterance of this study that he has given in his book. The volume is a judicious presentation of the subject, which does not make light of transgression, and at the same time clears away many of the ambiguities which frequently attach to terminology relating to sin.

The Problem of Evil in Plotinus. *By B. A. G. Fuller, sometime Instructor in Philosophy at Harvard University.*

Crown 8vo. pp. xx+336. Price 7*s.* 6*d.* net.

Publishers' Note

In this book the author makes an exhaustive criticism of the way in which one of the central problems of philosophy was treated by the most famous of the Neo-Platonists, and concludes that Plotinus, in spite of the wealth and ingenuity of his argument, never really escapes from the traditional dilemma —"either God is not justified or Evil is not explained."

Aberdeen Free Press.—This is a work of quite unusual significance. The author has called it "The Problem of Evil in Plotinus." It might as well have been called "A study of the problem of evil in general, with special reference to Plotinus."...Having stated the attempted solutions of the problem, the author proceeds to a review of the history of the development of the problem of evil in the history of Greek philosophy. The review is brief, but it may be characterised as a most lucid and competent bit of work...then there is a chapter presenting some general aspects of the Plotinian system...[followed by] chapters on metaphysical evil, on physical and moral evil, on matter as the principle of evil, and on the theory of emanation....On the whole we have not read for a long time a more satisfactory book on a philosophical problem, nor one which indicates a more masterly grasp of the evolution of philosophical thought, nor one which shows a higher power of masterly criticism.

NATIONAL ARCHIVES OF INDIA

Cambridge History of English Literature: Volume IX. From Steele and Addison to Pope and Swift. *Edited by A. W. Ward, Litt.D., P.B.A., Master of Peterhouse, and A. R. Waller, M.A., Peterhouse.*

Royal 8vo. pp. xvi+610. Price, in Buckram, 9*s.* net; in Half-Morocco, 15*s.* net.

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